Clinical and Diagnostic Studies of Acute Laminitis in Foals
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In this study acute laminitis in foals has been diagnosed in Mosul city, Iraq through an investigating of 14 male foals, 12-14 months old, accidentally engorged with carbohydrates diet and five clinically healthy foals of the same age range were considered as the control group. Affected foals showed different clinical manifestations. Blood samples from diseased foals show a significant increase (p<0.05) of packed cells volume, total leukocyte count, and Neutrophils in affected foals in compare to control group. There was considerable difference indicated in clotting factor indices. Significant (p<0.05) increase of Matrix Metalloproteinase is revealed in affected foals compared to control group. The histopathological features of affected foals show a hypertrophied disorganized structure like a teat shape of the secondary epidermal lamellae, while the tips of the primary epidermal lamella are separated from the basement membrane, however, the primary epidermal lamella cells are shapeless and collapsed from the basement membrane. Results of Radiographic examination indicated that the distal phalanx was significantly rotated from the hoof wall of the forelimbs, Furthermore, there is evidence of swelling of the heel region with the widening of coronary band accompanied by swelling of soft tissues and the distal phalanx slightly rotated from the hoof wall at the right and left hind limbs. It has been concluded that acute laminitis effectively and harmfully affects the sick animal, which may lead to a clear obstruction in walking movement and multiple health effects.

Keywords: Acute laminitis, Foals, Iraq.

Introduction
Laminitis, is an acute inflammation and degeneration of the sensitive laminae of equine hoof, occurs mostly from multiple causes such as, excessive eating of large quantities of lush green food, grain overload with consumption of high amounts of soluble carbohydrate, retention of placental membranes in mares, acute colic with continuous pawing. Moreover, overweight and Lameness with increased weight burden on one limb, besides excessive activity in hard-surfaced environments, and prolonged standing over several days when being transported, are considered an important causes of laminitis [1]. Acute systemic illnesses could also induce laminitis such as metritis, pleuropneumonia, and anterior enteritis, with severe diarrhea [2], corticosteroid-induced laminitis is also considered as one of the specific risk factors for the disease [3]. Laminitis occurs when the coffin bone or third phalanx being detached from the lamellae lining the hoof interior [4].

Acute laminitis is highly painful, possible career-ending, and maybe a lethal affliction to the epidermal and dermal laminae of digit that affects adult horses and ponies regardless of breed. The onset of pain as well as hotness, and higher digital pulses indicate the beginning phase of the disease, the critical phase continue until the displacement of the distal phalanx, which initiate the onset of the chronic phase [5,6].

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In general, it has been documented that the basic lesion of laminitis is detachment of the sensitive laminae of the third phalanx from the interdigitating laminae resulting in the dropping of the third phalanx through the hoof and ending up resting on the sole. However, the exact mechanism is unknown although it is speculated that a pain-hypertension-vasoconstriction cycle - develops in horses with acute laminitis [7].

Laminitis is a source of frustration for veterinarians since available information and perception of the pathophysiology of the disease is inadequate, thus restricting preventive and intervention initiatives. Additionally, economic losses for horse owners while trainers are emotionally stressed in observing the excruciating pain evident in diseased animals. As such, early diagnosis, remedial interventions, and biomechanical support of diseased feet are critical in alleviating the impacts of this overwhelming affliction since to date there are still no appropriate procedures for the prevention or rehabilitation of laminar damage [8].

It has been shown that the disease rarely affects foals younger than eight months old, however, the frequency might increase as the animals grow older [7]. Therefore, this current study highlights on acute laminitis diagnosed in foals.

Materials and Methods

Study animals
This study was conducted to examine 14 male foals, 12-14 months old, accidentally engorged with grains and other food containing a high concentration of soluble carbohydrates at Mosul city, Iraq. Five clinically healthy male foals of similar age were the assigned controls. Total clinical investigations were carried out of all the foals.

Blood samples
Ten milliliters (10ml) of blood were drawn from the jugular vein from each foal and a sample of 2.5mL was mixed with EDTA for evaluation of the total erythrocyte count (TRBC), hemoglobin concentration (HB), Packed cell volume (PCV), total platelet count (TPC), and total leukocyte count (TLC) (Hematology analyzer, Genex, USA). Furthermore, differential leukocyte count (DLC) was calculated employing Giemsa stain blood smears [9]. One more 2.5 milliliters of blood sample mixed with trisodium citrate (used plasma) was utilized to establish Fibrinogen time, prothrombin time, and activated partial thromboplastin time (Biolabo / France). Estimation of clotting time was made too based on Dayyal.[10]. The remainder of the blood was used for evaluating the serum Matrix Metalloproteinase (Elisa -MMP-2) according to the manufacturer’s instructions (Invitrogen, USA).

Histopathology
Two dead foals were subjected to histopathological examination. Hoof samples were taken and placed in a 10% formalin solution. The tissue models were cut to standard sizes and passed in ascending stages of 70% ethyl alcohol, then 90%, and finally, 100%. After that, samples were set in a 4% sodium sulfate solution and then in a 4% phenol solution. Small templates of wax were prepared then cut to stranded sizes and thickness and finally stained with eosin and hematoxylin [11].

Radiographic examination
The hoofs were cleaned off dirt and debris before performing a radiographic examination of the affected fore and hind limbs under exposure factors (70kv and 6 mAs). The radiographic examination included assessing the change in the distal phalanx and any abnormalities between it and the hoof wall. X-ray radiographs were taken in the Lateromedial view of affected limbs using the x-ray machine from Hitachi /Japan [12].

Statistical analysis
In the current study, the analysis of statistics was performed according to Leech et al.[13].

Results
Affected foals exhibited different clinical manifestations such as moving the front legs from the body (85.7%), Pain (78.5%), Increased intensity of the palmar digital artery pulsation (78.5%), Lameness (71.4%), excessive sweating (57%) and recumbence (11.7%) (Figure 1, Table 1).

Moreover, a significant rise (P<0.05) in body temperature, respiratory, heart rate, and capillary refill time in affected foals compare to control group was encountered. Furthermore, a substantial increase (P<0.05) of PCV, Total leukocyte count, and Neutrophils was recorded in affected foals compare to control group (Tables 2 and 3).
TABLE 1. Clinical manifestations of affected foals with acute laminitis.

<table>
<thead>
<tr>
<th>Clinical manifestations</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moving the front legs from the body</td>
<td>85.7</td>
</tr>
<tr>
<td>Pain</td>
<td>78.5</td>
</tr>
<tr>
<td>Increased intensity of the palmar digital artery pulsation</td>
<td>78.5</td>
</tr>
<tr>
<td>Lameness</td>
<td>71.4</td>
</tr>
<tr>
<td>Excessive sweating</td>
<td>57</td>
</tr>
<tr>
<td>Recumbence</td>
<td>11.7</td>
</tr>
</tbody>
</table>

TABLE 2. Clinical parameters in affected foals and controls.

<table>
<thead>
<tr>
<th>Clinical parameters</th>
<th>Control n= 5</th>
<th>Affected n= 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body temperature C</td>
<td>37.6± 0.22</td>
<td>39.4± 0.43*</td>
</tr>
<tr>
<td>Respiratory rate / min</td>
<td>13.6 ± 1.53</td>
<td>38.3 ± 4.21*</td>
</tr>
<tr>
<td>Heart rate/min</td>
<td>32.56 ± 0.82</td>
<td>61.3 ± 1.34*</td>
</tr>
<tr>
<td>Capillary refill time / Sec</td>
<td>1.2± 0.43</td>
<td>4.24± 1.57*</td>
</tr>
</tbody>
</table>

*Values are the mean ± standard error of the mean. * (P<0.05).*

TABLE 3. Hematological parameters in affected foals and control group.

<table>
<thead>
<tr>
<th>Hematological parameters</th>
<th>Control n= 5</th>
<th>Affected n= 14</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRBc x 10⁶</td>
<td>7.0±0.67</td>
<td>7.0±0.32</td>
</tr>
<tr>
<td>Hb mg/ dl</td>
<td>14.8±0.54</td>
<td>14.73 ±1.23</td>
</tr>
<tr>
<td>PCV %</td>
<td>34.4±3.84</td>
<td>45.11±4.3*</td>
</tr>
<tr>
<td>TLC x 10⁹</td>
<td>12.3±1.64</td>
<td>15.77±1.83*</td>
</tr>
<tr>
<td>Neutrophils /Absolute</td>
<td>5311±0.03</td>
<td>8046±0.56 *</td>
</tr>
<tr>
<td>Lymphocytes/ Absolute</td>
<td>5511±0.36</td>
<td>5342±0.64</td>
</tr>
<tr>
<td>Monocytes / Absolute</td>
<td>810±0.31</td>
<td>791±0.22</td>
</tr>
<tr>
<td>Eosinophils / Absolute</td>
<td>940±0.24</td>
<td>988±0.21</td>
</tr>
<tr>
<td>Basophils / Absolute</td>
<td>140 ±0.22</td>
<td>133± 0.66</td>
</tr>
</tbody>
</table>

*Values are the mean ± standard error of the mean. * (P<0.05). *
Significant difference has been documented in clotting factor indices, as a noticeable decline (P<0.05) in the TPC, while a significant rise (P<0.05) in mean platelet volume, platelet distribution width, clotting time, prothrombin time, activated partial thromboplastin time and Fibrinogen time were recorded. In addition, a significant increase (P<0.05) in Matrix Metalloproteinase of affected foals compared to the control group (Table 4).

Results of histopathological analysis of affected foals showed a hypertrophied disorganized teat-shaped structure of the secondary epidermal lamellae (SEL), which appeared empty caps. Moreover, the tips of the main epidermal lamella (PEL) were fully separated from the basement membrane, but the PEL cells were a shapeless mass and collapsed from the basement membrane (Fingers 2 and 3).

However, the outcomes of Radiographic examination indicated severely rotated distal phalanx from the hoof wall of the forelimbs and there was a slight periosteal reaction at the extensor process of the distance distal dorsal hoof wall more than proximal dorsal hoof wall distance about (8mm) that exhibited swelling of the heel region and all soft tissue above the hoof. Furthermore, there was evidence of swelling of heel region with a widening of the coronary band and also swelling of soft tissues as well as a distal phalanx slightly rotated from the hoof wall at the right and left hind limbs, Nevertheless, a measurement made of the image indicated a distance of the distal dorsal hoof wall (2mm) was more than proximal dorsal hoof wall with swelling of soft tissue at the heel region and area of the coronary band and above it. Fingers 4, 5 and 6.

### Table 4. Clotting factor indices and Matrix Metalloproteinase in affected and control foals.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control n=5</th>
<th>Affected n=8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total platelets count x10³</td>
<td>544.7± 15.33</td>
<td>258.4±22.13*</td>
</tr>
<tr>
<td>Mean platelets volume /fL</td>
<td>10.31± 0.6</td>
<td>14.3±3.2*</td>
</tr>
<tr>
<td>Platelets distribution width %</td>
<td>14.88± 1.2</td>
<td>21.3±3.41*</td>
</tr>
<tr>
<td>Clotting time / min</td>
<td>3.4± 0.72</td>
<td>5.4±0.34*</td>
</tr>
<tr>
<td>Prothrombin time /Sec</td>
<td>11.6±1.34</td>
<td>28.3±2.17*</td>
</tr>
<tr>
<td>Activated partial thromboplastin time/ Sec</td>
<td>51.4± 2.66</td>
<td>71.2± 5.22*</td>
</tr>
<tr>
<td>Fibrinogen time / Sec</td>
<td>15.48±6.43</td>
<td>45.76± 12.71*</td>
</tr>
<tr>
<td>Matrix Metalloproteinase (MMP-2) /ng/ mL</td>
<td>5.6±0.18</td>
<td>15.63± 2.74*</td>
</tr>
</tbody>
</table>

*Values are the mean ± standard error of the mean. * (P<0.05).
Fig. 3. A micrograph of hoof lamellar tissues of a foal with acute laminitis showing the tips of the primary epidermal lamella (PEL) are fully separated from the basement membrane, also, the PEL cells are a shapeless mass and collapsed from the basement membrane (red arrows). H & E stain. 10X.

Fig. 4. Latero-medial radiograph of the left hind limb of an adult foal, showing no signs of the distal pharynx being rotated from the hoof wall. However, there is evidence of swelling of the heel region with a widening of coronary band and swelling of soft tissues.
Discussion

Acute equine laminitis is considered an important and widespread disease due to its different clinical effects on diseased horses and which may sometimes lead to death [14, 15]. It has been shown that laminitis is rare in foals, especially those of young ages, and it is also not very common among adult foals [7].

Affected foals showed variable clinical manifestations which have been represented by some authors [16] and [17]. The pain associated with laminar degeneration causes the liberation of specific vasoconstrictors such as angiotensin II, catecholamines, and vasopressin which causes vasoconstriction of peripheral vessels, followed by a reduction in the blood flow to the hoof. The ischemia which results in combined with a micro...
thrombus formation, then the separation of the third phalanx from the hoof causes the rotation of the third phalanx within the hoof capsule, and to its vertical displacement within the hoof due to the weight bearing exerted via the third phalanx. Furthermore, the rotating of the third phalanx leads to the sole being pressed down or dropped, and which could cause the penetration of the sole by the point of the toe of the third phalanx [7,18]. The serum can accumulate in the space caused by the degenerated laminae and the third phalanx being displaced and there is a breakdown of the white line [19].

Non-structural carbohydrates (NSC), which comprise sugars and starches, are beneficial nutrients as they can be degraded to enable their absorption by the small intestine for storage as glycogen in the muscles and liver, and therefore, following their accumulation can be easily mobilized to generate energy. Although NSC exists in grasses throughout all growth phases, they are greatest when growth is fast following stressful periods. When horses ingest excessive levels of NSC, the carbohydrates move through the small intestine and spill over into the hindgut and undergo rapid fermentation, which induces higher acidity in the cecum, triggering various events, eventually resulting in decreased blood flow and nutrient supply to the foot, and causing laminitis. In addition, obesity and insulin resistance in horses could increase their susceptibility to posture-related laminitis [20,21]. Furthermore, Pollitt, 2008 [18] reported that the variations in the cecal mucosal barrier have been noted following carbohydrate excess. Acidosis in the cecal lumen with the lack of most gram-negative bacteria happens in the initial hours following carbohydrate excess. A systemic inflammatory reaction to this intestinal condition causes an increase in the vascular activity of the platelets and the white blood cells.

The origin of the laminar disease is in the vasculature and the acute lesion indicates ischemia with vascular injury and laminar edema [22,23]. However, Pollitt et al. [6], indicated that, in horses that developed acute laminitis, there was an increase of the pre-capillary as well as post-capillary resistance. The post-capillary resistance was considerably greater and seemed to be the main cause of the decreased capillary flow, but, simultaneously, the laminar interstitial fluid pressure increased and exceeded the capillary closing pressure, which compressed the capillaries, resulting in the reduction of blood flow.

The results of the study showed a significant increase in body temperature in diseased foals, which may be attributed to dehydration due to increased sweating as well as the pathological role of the endotoxins as reflected by the increased PCV rates. Moreover, Black et al. [24] and Constabl et al., [7], added that the high temperature may be the result of the liberation of endogenous pyrogens and their ability to cause a clear thermal response by directly affecting the thermoregulatory center of the brain.

Increased respiratory and heart rates of diseased foals reflected an acute systemic reaction to compensate for the disturbance to the blood circulation and blood flow in delivering the largest possible amount of blood to the affected hoof tissue. However, stress will occur due to increased adrenaline secretion causing vascular contractions and high blood pressure due to an impairment of venous and arterial pressure caused by peripheral vessels damage [25]. Significant rise was recorded in the total number of white blood cells with a significant increase in Neutrophils. This results agreed with Black et al., [24], who reported the elevation and infiltration outside the blood vessels in the first stage of acute inflammation. Furthermore, Hurley et al., [23], added that the migration of white blood cells into the interstitial tissues of the hoof as acute laminitis developed played a distinct role in pathogenesis.

The results of the current study indicated a significant difference in the clotting factor indices in affected foals compared to controls. It was documented that, Vascular damage with changes in laminar capillary permeability seems to remain a part of the pathogenesis. Following the commencement of laminitis, micro-thrombi exist in the capillaries of the diseased lamina, however, no study has determined whether the coagulopathy is the main or secondary cause of an ischemic attack [22,26]. Textor [27], explained the defect in the characterization of the thrombocytes activity during the inflammatory stages of acute laminitis in horses when the activity of platelets was recorded during the first six hours of inflammation when the tissue of the sensitive laminae of the hoof was destroyed, especially when the activity of inflammatory factors such as toxins was increased. Additionally, there was an increase in the activity of fibrin during the inflammatory stages to increase the sobriety of...
the blood clot, which was expected to take place in the blood vessels of the tissue of sensitive laminitis [14, 22].

The results of the current study indicated an increase in the values of matrix metalloproteinases (MMP). As MMP are pleiotropic enzymes that participate in the process of degrading extracellular protein and turnover. MMPs have a role in pathogenesis [28]. There is ample information on the role that MMPs play in the pathophysiology of human and animal diseases. Therefore, MMPs are now targeted for therapeutic intervention along with advances in developing MMP inhibitors [29-31].

Results of histopathological changes revealed different pathological tissue changes as a result of acute inflammation, most of those changes were also mentioned by Van Eps, Pollitt [32], who found that the transverse sections of the dorsal hoof wall lamellae were considerably greater in length than normal, while several secondary epidermal lamellae were separated from the main lamellae and were present as spherical or ovoid, discrete islands secluded in the lamellar dermis. The lamellar basement membrane was unbroken. Moreover, others, de Laat et al. [33], indicated that the important potential relevance concerning acute laminitis is, the construction and ultimate strength of the resultant lamellar interface could be highly improved by strategically minimizing the mechanical displacement within the acute phase of laminitis. On the other hand, Laskoski et al. [34], concluded that the lamellar tissue was able to re-arrange quickly after an episode of acute laminitis. Despite the absence of histopathological indications of existing acute laminitis for several days, there was a noticeable disturbance of the lamellar architecture.

It has been documented that, radiographic assessment is the sole imaging modality that is as valid as the physical assessment. When applying traditional and digital radiography, methodology and perceptions are homogenous. Standard perceptions from the lateral to medial, dorsal palmar, and 45° dorsal palmar projections must be carried out routinely. Outcomes need to be subjected to assessment concerning the rest of the clinical picture, which should take into consideration the stage of the disease and particularly, the speed at which the coffin bone changes its position in the hoof capsule. It is significant to realize that the radiographic study is representative of a static image of a dynamic model and disease. It is very beneficial in combination with the clinical assessment during serial sessions [12]. The results of the current study agreed with Sherlock and Parks, [35], who found that the Radiographic evaluation had been altered from merely recording the measurement of the coffin bone’s rotation, but this remains a preferred choice because the technique is relatively standardized and familiar. The palmar angle of the third phalanx, horn-lamellar (H-L) zone width proximal and distal, extensor process-coronary band distance, sole depth at the tip and wing of the distal phalanx, and soft tissue are other objective parameters currently evaluated. Furthermore, the coffin bone integrity is similarly assessed for proliferative or wide-ranging changes, relative density, and porous or pathologic fracture. Finally, the development of radiographic changes in the long term is a significant consideration.

Conclusion

It has been concluded that acute laminitis harms foals and may lead to chronic impairment of the animal’s locomotor system.

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Competing Interest Statement

In the current study, the authors certify and acknowledge there is no Competing interest statement.

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References


دراسة سريرية وتشخيصية للالتهاب الحاد في الصفائح الحساسة في الامهار
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كلية الطب البيطري , جامعة البصرة , مديرية الطب البيطري , كرمين , سليمانية , كردستان العراق

تم تشخيص التهاب الصفائح الحساسة الحاد للامهار في مدينة الموصل / العراق , في بحث تم إجراؤه لفحص 14 ذكرًا من الامهار ، تراوح أعمارهم بين 12 و 14 شهرًا ، احتقت بطريق الخطأ بالكربوهيدرات. تم اعتبار خمسة من الامهار السليمة سريرياً من نفس الفئة العمرية كمجموعة سيطرة وضعت. أظهرت الامهار المريضة علامات سريرية مختلفة. مع زيادة معنوية في معدلات ترسيب كريات الدم الحمر وزيادة معنوية في العدد الكلي لخلايا الدم البيضاء والحالات في الامهار المريضة بالمقارنة مع مجموعة السيطرة فضلا عن الاختلاف في معدلات عامل تخثر الدم. كما لوحظ زيادة معنوية في معدلات حمية القلب المبالة للبروتين المعينة في الامهار المريضة بالمقارنة مع السليمة. أظهرت نتائج الفحوصات النسيجية المرضية بيئة غير منظمة ومنظمية بشكل يشبه الحلمات لصفائح البشرة الثانوية في حين تم ملاحظة انفصال اطراف صفيحة البشرة الأولية عن الغشاء القاعدي. مع ذلك ، فإن خلايا الصفيحة الأولية كانت عديمة الشكل والتهابات من الغشاء القاعدي. أشارت نتائج الفحص الشعاعي إلى دوران السلامية القاسية بشكل كبير من جدار الحافر لأطراف الأمهار علواً على ذلك ، لوحظ تورم منطقة الكعب مع انباط نافذة التهابي مصحوبة بتورم الأنسجة الرخوة مع استدامة سبيكة للسلامية القاسية من جدار الحافر في الأطراف الخلفية اليمنى واليسرى. استنتج من هذه الدراسة أن التهاب الصفائح الحساسة الحاد يؤثر بشكل فعال وضرر على الحيوان المريض ، مما قد يؤدي إلى إعاقة واضحة في حركة المشي وتأثيرات صحية متعددة.