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# thesis entitled COMPUTER-ASSISTED THREE-DIMENSIONAL GAIT ANALYSIS OF AMPHOTERICIN-INDUCED EQUINE CARPAL LAMENESS

presented by

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## COMPUTER-ASSISTED THREE-DIMENSIONAL GAIT ANALYSIS OF AMPHOTERICIN-INDUCED EQUINE CARPAL LAMENESS

Ву

John Gerard Peloso

## A THESIS

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in partial fulfillment of the requirements
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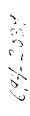
## **ABSTRACT**

## COMPUTER-ASSISTED THREE-DIMENSIONAL GAIT ANALYSIS OF AMPHOTERICIN-INDUCED EQUINE CARPAL LAMENESS

By

#### John Gerard Peloso

Using six clinically sound horses trotting at 4.0 m/s on a treadmill, motion was captured using a computerized three-dimensional motion analysis system before and 9, 16 and 23 days after lameness induction to determine: the efficacy of threedimensional computer-assisted image analysis for objective lameness evaluation in horses and the quantitative variables which significantly describe this lameness. The dorsal vertical distance travelled by the head (HE) and withers (WE) was increased significantly during all lameness measurement periods when coupled to the sound forelimb. HE but not WE was decreased significantly during all lameness measurement periods, when coupled to the support phase of the lame forelimb. Computer determinations of stride length, stance phase, forelimb abduction, carpal and fetlock range of motion did not significantly characterize the lameness. It was concluded that three-dimensional computer-assisted image analysis could be used for objective lameness evaluation in horses and that head and withers excursion were reliable variables for the assessment of equine carpal lameness.



## **DEDICATION**

To my parents Donna Marie and Dino Ferdinand Peloso for your attention to the essence of life and your love while you shared these philosophies.

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## I. INTRODUCTION

Abnormalities of the equine musculoskeletal system are the leading cause for performance loss in the equine athlete<sup>1-3</sup> and the main reason why equine veterinary services are requested.<sup>4</sup> It has been recommended that research on the equine musculoskeletal system assume a high priority <sup>2,5</sup> because lameness has been documented as the most significant factor causing wastage among young racehorses.<sup>1-3</sup> Despite these recommendations, few objective lameness studies have been performed on horses.<sup>6-8</sup>

Locomotion has been defined as all the biomechanical events that occur while an animal or person moves from one place to another in a controlled and deliberate manner. Biomechanics can be subdivided into statics, the effects of forces on bodies at rest, and biodynamics, the study of bodies in motion with reference to the forces and masses involved. Biodynamics is further subdivided into biokinetics, the study of forces acting on bodies to produce motion, and biokinematics, the geometric description of motion without reference to forces. A comprehensive biomechanical evaluation includes measurements of relative or absolute body segment motion changes (biokinematics), a study of forces acting on bodies to produce these motions changes (biokinetics) and an analysis of the

electrical activity of the muscle, (electromyography)<sup>11</sup> in order to document which muscles are responsible for the specific forces and motion noted at a given point in the stride cycle.

This project is concerned exclusively with biokinematics, the temporal and geometric characteristics of motion without regard to the associated forces or muscles involved. Extensive reports on the different technologies available for quantitatively assessment of motion have been published.<sup>9,12-15</sup>

The main objective in this study was to determine if computer-assisted three-dimensional video gait analysis technology currently used in human medicine could be used to objectively evaluate lameness in horses. Our specific objectives were: 1. To determine what quantitative variables could be used to assess lameness in the horse and 2. To determine which of these variables most reliably provide assessment of equine carpal lameness.

#### II. LITERATURE REVIEW ON LAMENESS ASSESSMENT

## A. Development of Motion Analysis

Review of the literature suggests that the earliest investigation into quadruped limb motion was likely conducted by Aristotle.<sup>16</sup> The first reported study of photogrammetry (the science of obtaining reliable measurements by means of photography) was conducted by Muybridge in 1872. Muybridge, a photographer

in California, was sponsored by Leland Stanford to photograph the various phases of a horse's gait to determine the most scientific method for training a race horse, and so began the age of quantitative motion assessment. A series of 24 cameras, with exposures of 2/1000 of a second, were set at right angles to the track and line of motion. The horse broke a thread as it trotted by and tripped the shutter in each camera.<sup>17</sup> In an 1878 *Scientific American* article, Muybridge indicated that the vertical lines in the background of his photographs are separated by 28 inches. Knowing that his subjects trotted at a speed of 2 minutes and 24 seconds per mile, it allowed the captured motion to be analyzed. He proved that the walking horse always has two feet on the ground, and for a brief moment three. He revealed that the stride length of a trotting horse increased 4 fold compared to a walk and while trotting, the horse is completely off the ground for half the length of the stride.<sup>18</sup>

When the first motion picture camera was introduced in 1923, photogrammetry became a viable research tool. 19 In 1939, quantification of human athletic performance was improved by using the multiplier technique. 20 As sprinters travelled perpendicular to the cameras optical axis, motion was quantified by placing a bar of known length in the filming sequence. Knowledge of this length combined with the measured length of the corresponding image provided a single multiplier coefficient and allowed quantitative measurements to be derived from the

films.<sup>20</sup> In 1960 Kaemmerer used photogrammetrics to improve the description of limb flight in horses by attaching lights to the distal aspect of their limb.<sup>21</sup> Though these changes helped simplify the work involved in quantifying the images seen, motion assessment was still limited to a plane perpendicular to the cameras optical axis. In a 1967 human kinematic research trial, Noss used three cameras located precisely along three orthogonal (mutually perpendicular) axes to overcome the problem of perspective error. He believed that perpendicular optical axes with a common point of intersection could be used to derive accurate three dimensional position data.<sup>22</sup> Thirteen years passed before this simple method, devised to correct for camera perspective errors, was proven to be invalid.<sup>23</sup>

In 1972 Fredricson and Drevemo described a system for quantitative analysis of equine locomotion using high speed film in order to derive kinematic data. They also provided a technique for three-dimensional analysis of joint movement while filming Standardbreds at speed. Correcting problems associated with camera perspective errors, precise camera orientation before filming and hours of pre-test set-up time were rectified in 1971 by Abdel-Aziz and Karara. These ideas were modified to include orientation constants from the camera interior and then developed for human clinical biomechanics assessment in 1981 by Dr. Jim Walton as part of his Ph.D. project at Pennsylvania State University. By using a technique known as direct linear transformation (DLT), the above mentioned

authors removed all restrictions in camera placement and relative orientation.

In recent years, advances with motion assessment have included the development of cameras with higher speeds and better image resolution, fully digitized photogrammetric systems, improved image processing and analysis. As well, computer software packages with integrated image processing and three dimensional graphic systems have been designed to provide real time data acquisition.<sup>30</sup>

## B. Alternate Motion Analysis Techniques

#### 1. Visual Lameness Assessment

In 1985, the reliability of observational kinematic gait-analysis was evaluated using a three point scale on 15 children that wore a bilateral knee-ankle-foot orthoses due to lower limb disabilities.<sup>31</sup> Three experienced and highly trained clinicians, who had received prior training on the three point scale, evaluated these children on two occasions. The second ratings occurred thirty days after the first. In this study, self-agreement occurred 69% of the time and between-rater agreement occurred, 67.5% of the time. It was concluded that substantial variability exists among and between trained specialists in the visual assessment of lameness, making this technique unreliable.<sup>31</sup>

Similarly, visual assessment techniques were used in ponies and shown to be

unreliable. Ponies that had been previously evaluated on a force plate received an intratendinous injection of collagenase to create inflammation in the superficial digital flexor tendon. After nine months, the force plate measurements were repeated and were combined with clinical examination for lameness by an experienced clinician. All three groups were rated as clinically sound by the clinician but the force plate was able to distinguish between performances that had returned to normal and those which had compensated and therefore appeared sound.<sup>32</sup> Consequently, measurement systems which generate quantitative information that can be analyzed are preferred over visual assessment techniques.<sup>9</sup>

## 2. Kinematic Analysis Systems

## a. Cinematography and Videography

The techniques available include single camera photography, multiple camera photography, 8mm movie cameras<sup>33</sup>, high-speed movie projectors and video cameras. Though limited quantitative information can be retrieved from these systems, they provide a permanent record that can be subjectively evaluated.<sup>13</sup>

A 16mm high-speed camera is the system most frequently used in equine gait analysis laboratories. The advantages of a 16 mm camera are its ability to provide a permanent record and yield large amounts of information without altering the

movement of the horse. However, because the film plane must be perpendicular to the line of travel of the horses,<sup>7</sup> and course markers have to be exactly spaced, preparation times are excessive. Additionally, errors caused by moving skin markers,<sup>15,34,35</sup> delays in film processing,<sup>13</sup> equipment expense,<sup>7,15</sup> and the labor involved in film analysis<sup>7,15</sup> further complicate this technique. Two or more high-speed cameras may be used from different vantage points to photograph the horse, but this practice is limited to laboratories where more than two or three strides can be collected.<sup>36</sup> Three dimensional analysis is possible using high speed cinematography, but is usually done in two dimensions<sup>36,37</sup> because cameras need to be synchronized making set up complex.<sup>15</sup> An alternate method was used to collect more than three strides of data per filming session. This involved following the subject around a race track with a camera mounted to a moving vehicle.<sup>25,27,38</sup>

The advantages of videography are similar to high speed cinematography; they provide a permanent record, a great amount of information and data collection does not alter the normal movement of the horse. They are also easier to operate, allow immediate play back and the videotapes can be re-used. Unfortunately video cameras have poor image resolution. In an effort to improve resolution, cameras may be moved closer to the subjects but have their field of view decreased. These video cameras are expensive, and similar to all situations where

targets are fixed to moving subjects, errors can occur due to marker movement.9

## b. Automated Optical Systems

Optical systems capable of automated movement analysis are available. Improved camera perception of target location, when compared to 16 mm cinematography, is facilitated by using infra-red light emitting diodes or retro-reflective colored prisms. Transmitted light is perceived by cameras and sent to a digitizer which rapidly converts the position of markers on the subject into X & Y coordinates. The computer accepts the X,Y coordinates from the digitizer and assimilates the data for analysis. Since light emission is essential for target recognition, these systems are restricted to the laboratory where extraneous sources of light are omitted.<sup>14</sup>

#### c. Electrogoniometry

In 1977 Adrian et al used electrogoniometers (electrical instruments) to examine angle changes of the metacarpophalangeal joint in four Thoroughbreds. <sup>39,40</sup> Electrogoniometry consists of an elgon (electrogoniometer), an amplifier and a recorder.<sup>37</sup> The elgon is a potentiometer that functions as an electrical protractor. Initially, the elgon is calibrated on a protractor and mounted over the center of rotation of the joint to be evaluated. The chassis and electrical leads are then taped to the leg and connected to an amplifier attached to the subject. Changes

in joint angles are recorded as the horse moves at the selected gait. The goniograms may be analyzed manually or digitized and analyzed by a computer. Goniometry has been used in the normal<sup>41</sup> and clinically lame horse.<sup>39</sup> It has been used to assess motion of the fetlock, hock and carpus,<sup>41</sup> and provides direct, continuous recording of joint motion from horses moving at all gaits. It has the disadvantages of being difficult to accurately locate and secure over the center of rotation of the joint, particularly in the proximal aspect of the limbs due to skin movement. Because few joints move in a single axis, these devices may restrict normal joint movement due to the planar nature of the chassis and the methods used to secure it to the limb.<sup>14</sup>

## 3. Kinetic Analysis Systems

## a. Force Platforms

Force platforms were originally developed for the horse in 1976 by Pratt and O'Connor,<sup>42</sup> and have since become the standard for kinetic measurements. They are composed of a hardened aluminum upper surface with underlying strain gauges or piezoelectric crystals which produce force measurements. A force platform is designed to quantify the magnitude, direction and duration of a ground reaction force (GRF). The GRF is comprised of three forces, the vertical forces ( $F_z$ ),: cranio-caudal forces( $F_v$ ) and medio-lateral forces ( $F_x$ ).<sup>8</sup> The variability inherent in

locomotion has caused people to recommend a minimum of 25 trials per test period for humans and a minimum of 30 for horses.<sup>32</sup> In horses, an average of one in 20 passes by the force plate delivers an adequate foot strike<sup>32</sup> making this system very time inefficient.

## b. Equine Kaegi Gait Analysis System

The Kaegi Gait Analysis System has electromagnetic field sensors below its upper surface. These sensors are connected through a series of fluid-filled tubes to piezoelectric crystals. The hydraulic impulse created by the weight of the horse, is translated into an electric signal by the piezoelectric crystal which is proportional to the vertical force from the limb.<sup>43</sup> This system is limited in its usefulness since it measures vertical forces only<sup>12</sup> and cranio-caudal horizontal force have proven to be useful in lameness diagnosis.<sup>8</sup>

## c. Accelerometers

Accelerometers are small device that attach to a body or limb segment to measure its acceleration. A piezoelectric crystal detects and quantifies the acceleration of movable parts in the accelerometer and send a signal proportional to that acceleration. By selecting a uni,bi or triaxial accelerometer, one two or three axes of motion can be evaluated. Accelerometers do require cables to be attached to the horse and therefore may interfere with normal motion.

#### d. Instrument Shoes

Instrument shoes (force shoes) capable of measuring vertical, or vertical, medio-lateral and cranio-caudal force measurements are available. These shoes use one or more piezoelectric force transducers attached to a racing shoes to detect force changes. Cables from the transducers extend from the shoes up the limb to instruments necessary for modifying and storing the signal. Force shoes can measure consecutive strides of the trot, canter or gallop and enable the collection of data from a perfect "strike" every stride. They can be attached to all four limbs to obtain a more complete force profile while a lighter (aluminum) design has allowed gait evaluation at high speeds to be performed safely. They are currently not used as a clinical tool since the shoes have to be fitted and nailed to the foot for accuate data acquisition.

#### C. Assessment of the Lame Horse.

Though quantitative reports describing the lame horse are uncommon,<sup>6,44</sup> it is hypothesized that the symmetric nature of animals and humans allow asymmetries in individual gait to be identified as lameness.<sup>9</sup> This hypothesis was tested by Drevemo et al using 30 clinically sound trotting Standardbreds.<sup>45</sup> It was demonstrated that limb movement variations in any particular horse were very small when assessing stride length, and the duration of stride, stance, swing and

propulsion.

Unilateral lameness in the trotting horse is subjectively thought to produce changes in both the timing (eg stance phase) and distance in limb movement (eg stride length). A stride is defined as a cycle of locomotor events which begin with the stance phase of a particular limb and ends with the subsequent stance phase of that limb. Stride length is conventionally defined as the horizontal distance covered along the plane of progression during one stride. In the normal horse, stride length increases linearly with velocity through the complete velocity range. Stance phase is defined as the time period when the limb is in contact with the ground. The appears that potential stride modifications between subjects are inconsistent, for example; when comparing stance phase times between sound and lame limbs, published reports have shown it to be unaffected. decreased and increased.

Changes in the vertical position of a horses head and neck, which together comprise approximately 10% of their body weight,<sup>52</sup> has been the standard in forelimb lameness evaluation. In the original <u>Lameness in Horses</u> by Dr. Adams he states, "As a result of lameness in a forelimb, the head will drop when the sound foot lands.<sup>53</sup> These beliefs are summarized in the fourth edition of Stashak's <u>Adams' Lameness in the Horse</u>: "Typically for the forelimbs, the horse drops his head down on the sound limb and raises it up to a more normal height

on the affected side" and "At a trot the lameness becomes obvious and some head and neck lifting occurs when the affected limb hits the ground".<sup>54</sup> Explanations for the change in head and neck position include an attempt to reduce the weight bearing on the affected limb<sup>54</sup> and an effort to shift the center of gravity caudally, away from the lame forelimb, as the head is elevated.<sup>6</sup>

Several quantitative force platform analyses have been conducted in unilateral models of forelimb lameness.<sup>8,55,56</sup> Changes associated with compensatory unloading of the lame limb have been uniformly demonstrated. The injured forelimb of walking or trotting horses have decreased vertical forces, decreased cranio-caudal decelerative horizontal forces, with no change in the cranio-caudal accelerative forces. The contralateral sound forelimb has shown no change in the vertical forces, increases in the cranio-caudal decelerative forces and decreases in the cranio-caudal accelerative forces.<sup>8,56</sup> Contact time (stance phase) averaged around 242.8 msec for both the sound and lame limb and was not affected by the induction of lameness.<sup>8</sup> Comprehensive kinematic studies on the lame horse are not available.<sup>6,44</sup>

## D. Equine Arthritis Models

A diarthrodial or synovial joint contains the ends of two articulating surfaces of bone that are covered with hyaline cartilage, a soft tissue joint capsule that envelopes these structures and synovial fluid that fills the joint space. The joint capsule has a fibrous outer layer and an inner synovial layer. This synovial layer lines the joint space and contains synoviocytes which secrete synovial fluid.<sup>57</sup>

Arthritis, or osteoarthritis, is literally translated to mean inflammation of the joint. The expression degenerative joint disease (DJD) is now used synonymously with all forms of osteoarthritis,<sup>58</sup> and represents a group of disorders characterized by alteration of the articular cartilage and accompanying changes in the bone and soft tissues of the joint.<sup>58</sup>

Though use-trauma is considered to be the most important initiating factor in DJD,<sup>59</sup> the inciting cause for DJD is unknown and includes the following theories;<sup>58</sup>

- 1. Primary soft tissue inflammation.
- 2. Cyclic fatigue damage to the collagen network in cartilage.
- 3. Trauma causing microfractures in the subchondral bone which subsequently stiffens and results in decreased shock absorption and cartilage degeneration.

Primary synovitis is characterized by inflammation of the synovium without articular cartilage damage and results from physical or chemical damage to the soft tissue as would occur by repeated overextension. Secondary synovitis occurs as a result of trauma to or degeneration of the articular cartilage or subchondral bone. The interaction between synovium and articular cartilage is complex and incompletely understood, but it is known that structural and functional

alterations in one tissue intimately affects the other. 60

Osteochondral fractures of the equine carpus are common in racehorses, <sup>61-64</sup> and in particular, those horses that train and race on a dirt track. <sup>62-65</sup> Horses having carpal arthropathy may be temporarily or permanently removed from training or racing, but accurate diagnosis and appropriate therapy is associated with a favorable prognosis for return to racing with figures of 79 % in one report and 80 % in another. <sup>62,66</sup> In this project, arthritis was induced in the middle carpal joint because the carpus is associated with a high incidence of injury, <sup>62,65</sup> and has been the major focus of objective arthritis and lameness relate research. <sup>55,67-72</sup> To develop a greater understanding of equine arthritis and to quantitate different treatment modalities, both physical and chemical regimens have been used to induce joint inflammation.

Arthritis has been physically induced by creating osteochondral fractures on the dorsomedial aspect of both radial carpal bones,<sup>75</sup> and by creating partial <sup>67-69</sup> and full thickness defects in cartilage, then subjecting these horses to exercise.<sup>8,68,69</sup>

Osteoarthritis has been chemically induced using several different agents.

Using 0.5mls of Freund's complete adjuvant, Hamm et al produced a carpal arthritis to study the effects of an intra-articular medication. Arthritis was characterized by local swelling, pain and an increase in synovial protein.<sup>70</sup> Hurtig

et al injected 10 mg of autogenous femoral trochlear cartilage into the left middle carpal joint and then exercised horses, anticipating a more authentic arthritis. The model was characterized by a mild synovial effusion, capsulitis and transient lameness. Alternatively, 50 mg of sodium monoiodoacetic acid has been used to produce articular cartilage degeneration, periarticular swelling and synovial effusion. Firth injected E. Coli lipopolysaccharide into the middle carpal joint of 6 ponies and created articular effusion, pain on flexion, increases in synovial rotein, total leukocyte and neutrophil numbers and a lameness that lasted 36 hours. Amphotericin-B was selected to induce arthritis in this project because the polyene antibiotics, amphotericin-b<sup>73</sup> and filipin <sup>74</sup>, had been used previously to induce synovial arthritis in horses.

Amphotericin-B functions as an antifungal<sup>76-78</sup> by forming an irreversible bond to ergosterol, the principal sterol in the cell wall of fungal organism.<sup>78</sup> Leakage of potassium ions and other intracellular components result in the eventual lysis of sensitive fungi. Amphotericin-B binds less aggressively to cholesterol, the principal sterol of mammalian cell membranes, and therefore has a reduced effect on animal tissues.<sup>78</sup> Filipin-induced arthritis has been observed to disrupt synovial cell lysosomes and increase acid phosphatase activity in synovial fluid thereby producing an inflammatory arthritis.<sup>73</sup> Amphotericin-B has also cause a marked and persistent increase in acid phosphatase activity in synovial fluid and it is

theorized that, like filipin, the mechanism of induced arthritis is due to disruption of synovial cell lysosomes.<sup>74</sup> These effects are beneficial since synovial lysosomal enzymes, rather than damaged articular cartilage, are the major contributor to the lysosomal content of equine synovial fluid.<sup>73</sup>

## III. PRINCIPLES OF MOTION ANALYSIS

#### A. Introduction

In this project, data acquisition and reduction utilized established biokinematics, photogrammetrics, anatomy, science, mathematics, physics, optics and sophisticated computer technology and software packages. The sections that follow are provided to give the reader a better understanding of the pertinent aspects of these disciplines as they relate to this thesis. More exhaustive explanations are available in the references provided.

## B. Orthogonal Coordinate System

A reference coordinate system, including an origin and coordinate directions, is required to measure the position of bodies in space. All locations in the reference system indicate a position relative to the origin while movement reflects changes in position closer to, around, or further away from the origin. Since a reference coordinate system should span the space of interest, three non-coplanar coordinate directions need to be selected.<sup>79</sup> Calculations are greatly simplified

when the axes are orthogonal<sup>80</sup> (mutually perpendicular) and therefore a right handed orthogonal coordinate system with axes labeled X,Y,Z was utilized. This orthogonal system is useful when examining vectors<sup>81,82</sup> since their components, or the magnitude of the vector in the X,Y, or Z direction, is simply the projection (the cosine) of the vector on the coordinate axes.<sup>10</sup>

## C. Direct Linear Transformation

Direct linear transformation (DLT) requires advanced filming of at least six non-coplanar control points to generate a set of twelve equations per camera. This a priori filming of the non-coplanar points, known as calibration, provides control points that are measured values from a previously assembled and exactly measured calibration structure. Each set of DLT equations contain terms that define the image and object coordinates of each control point as well as eleven calibration coefficients. These coefficients contain absolute information regarding camera orientation, lens to image plane distance, and the linear components of lens and image distortions. Nowledge of the known object and measured image coordinates for each control point provide the user with an overdetermined system of twelve equations with eleven unknowns. A linear least squares technique is used to approximate the values of the calibration coefficients. 28,78

At least two image planes (cameras) are needed to yield precise estimates for

the location of a point in three dimensional space, so a second set of DLT equations are generated for these control points as viewed by a second camera. <sup>28,76,78</sup> Again using the known object (calibration structure) and measured image coordinates of each control point, calibration coefficients can be approximated for this second camera.

Since each object point has a unique (X,Y,Z) location in space, these two sets of equations provided by two camera perspectives, can be combined provided they are time matched. This combination of equations is known as the intersection equation and is analogous to the intersection of two principal optical rays at the location of the object at point 'O' (Figure 1).<sup>29,81</sup>

The three-dimensional spatial coordinates of unknown targets placed in the space encompassed by the calibrated structure are determined by way of a direct linear transformation using the four (or more) DLT equations (two from each camera) and the two dimensional image coordinates established from each image (camera) plane.<sup>81</sup> These four equations with three unknown spatial coordinates are again overdetermined and a least squares technique is again used to provide an estimate of their location. This estimate can be improved by increasing the number of cameras used at different vantage points.<sup>29,79,81</sup>

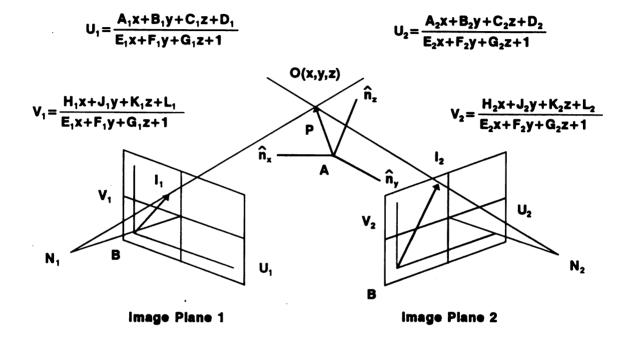


Figure 1. The geometric representation of the intersection equations used to determine the unique three dimensional location (x,y,z) of point O in object space. Image plane 1 and 2 represent the perspectives of each camera to object O. The object reference system has origin A and coordinate directions  $n_x, n_y, n_z$  while the camera reference systems has origins B and digitized coordinate axes U and V with the values of  $I_1$ ,  $(U_1, V_1)$ , and  $I_2$ ,  $(U_2, V_2)$ . The values the 11 camera coefficients, namely  $A_1$  to  $L_1$  and  $A_2$  to  $L_2$  are known and are determined during calibration. The values of  $U_1, V_1$  and  $U_2, V_2$  are also known and are determined from the digitized coordinate axes U and V. Therefore, the values x,y,z are the remaining unknowns and can be determined by solving the four equations shown. <sup>29,81</sup>

## D. Retro-reflection

The exact location of limb and body segments are facilitated by attaching light-emitting targets to exercising subjects. Targets can be covered by a retro-reflective tape and therefore intensify their identification. With mirror reflection, a beam of light is reflected at an angle equal and opposite to the angle of incidence. With diffuse reflection a beam of light is reflected in all directions, and reduces the intensity of light transmitted. By using a retro-reflective surface on targets two optical principles can be exploited, namely cube corner and spherical bead lens reflection. These phenomenon cause reflected light to be transmitted directly to the original source of light and provide a reflective efficiency 50 to 3000 times that of white light. When target brightness is sufficient, supplementation through electrical wires, which can interfere with normal gait. Is not needed.

## VI. MATERIALS AND METHODS

#### A. Horses

Six adult mixed-breed horses [mean( $\pm$ SEM); body weight 388( $\pm$ 8.3 kg), age 3.2( $\pm$ 0.64 yrs)] were used in this study. All animals were maintained on a 1 acre pasture for 30 days prior to experiments and were vaccinated against equine influenza, rhinopneumonitis, and tetanus. All animals were dewormed with an anthelmintic and shown to be sero-negative for equine infectious anemia. The

horses were determined to be suitable subjects based on the results of a pre-study health examination. The health examination included a rectal temperature, pulse, respiratory rate, complete blood count and serum chemistry. A lameness examination, including radiographs of both carpi, (including a flexed lateromedial, dorsolater-palmaromedial oblique and dorsomedial palmarolateral oblique) was also performed. The horses were trained to walk for 1 minute at 1.8 m/s and to trot for 2 minutes at 4.0 m/s once a day for three days on a high speed treadmill<sup>a</sup>. Horses exhibiting lameness either during the initial examination or after treadmill training were removed from the study. During the acclimation and measurement phases of the study all animals were housed in individual stalls.

#### B. Experimental Design

Baseline motion data was recorded on both computer disk and video tape on day one (Table 1). Following this, all animals received an intra-articular injection of 20 mg of amphotericin-B<sup>b</sup> in 5 mls of sterile water in the left middle carpal joint. This was repeated using 25 mg of amphotericin-B in 5 ml of water on day 3 and day 5 of the protocol. Phenylbutazone<sup>c</sup> at the dose of 2.2 mg/kg was given orally once, after the first amphotericin-B injection, to control animal discomfort.

a. Mustang 2000, Kagra AG, Fahrwangen, Switzerland.

b. Fungizone, ER Squibb & Sons Inc., Princeton, NJ.

c. Butazolidin Paste, Coopers Animal Health Inc., Kansas City, KS.

Additionally, 0.1 mg/kg of butorphanol tartrate<sup>d</sup> was given intramuscularly every 6 hours for the first 24 hours or longer to effect. The butorphanol injections were repeated on days 3 and 5 of the protocol. Motion data was recorded on both computer disk and video tape on days 9, 16, and 23. At the end of the study each horse was euthanized using an intravenous barbiturate overdose in accordance with the guidelines established by the Animal Care Committee at Michigan State University.

Table 1. Protocol followed to induce arthritis and evaluate lameness.

Day	Procedures		
1	Ma*, Video⁺, Ampho*, Pbz <sup>@</sup> , Butor*		
3	Ampho, Butor		
5	Ampho, Butor		
9	Ma, Video		
16	Ma, Video		
23	Ma, Euthanasia		

MA = Computer motion data acquired Video = Video camera images collected

Ampho = Intra-articular amphotericin-B injected

Pbz = P.O. phenylbutazone administered

Butor = I.M. butorphanol injected

d. Torbugesic, Fort Dodge Laboratories, Fort Dodge, IA

# B. Measurement Technique

# a. Room Design

All data acquisition took place at Michigan State University Equine Performance Center in an area depicted in Figure 2. Four 60 hertz cameras\* and their accompanying light sources were placed symmetrically around the treadmill. The cameras were arranged to capture the images from reflective spheres on trotting horses, then co-axial cable ported the information to the video analyzer\*. These images were converted, by the video analyzer and a computer work station\*, to four files of two dimensional data. A portable video camera\*, positioned at V1 and V2 (Figure 2), was used to videotape each trial for subjective evaluation.

e. T1-23A CCd, NEC Corp., Tokyo, Japan.

f. VP320, Motion Analysis Corp., Santa Rosa, CA.

g. Sparc Station 1, Sun Microsystems Inc., Mountain View, CA.

h. SK-F200K, Toshiba Corp., Denver, CO.

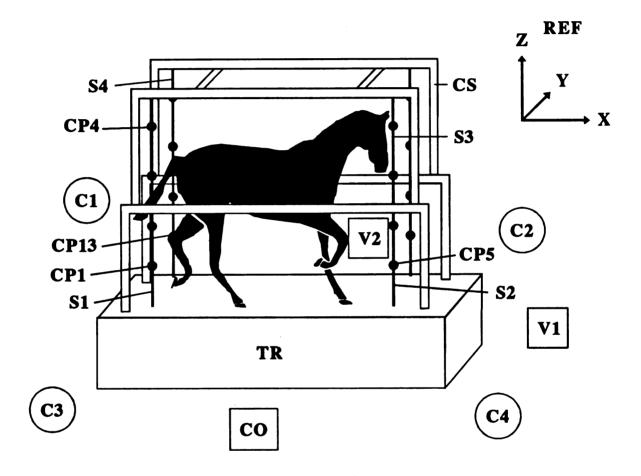


Figure 2. Topographic arrangement of laboratory used for data acquisition. Equipment used included a treadmill (TR), computer (CO), computer cameras (C1,C2,C3,C4), calibration strings, (S1,S2,S3,S4) calibration stand (CS), target control points (CP1, CP4, CP5, CP13), object reference coordinate directions (REF) and positions where video camera images were collected for subjective evaluation (V1, V2).

### b. Clinical Determination of Lameness

As horses trotted on the treadmill at 4 m/s, sixty seconds of videotape was collected for each horse from point V1 and V2 (Figure 2). Two independent observers evaluated the videotapes and graded horses on a scale of 1-5 using an accepted lameness scale (Table 2).<sup>84</sup>

Table 2. Lameness scale from the AAEP Definition and Classification of Lameness.<sup>84</sup>

Definition: Lameness is a deviation from the normal gait or posture due to pain or a mechanical dysfunction.

#### Classification Grades:

- 1. Difficult to observe; not consistently apparent regardless of circumstances (i.e. weight-carrying, circling, inclines, hard surfaces, etc.)
- 2. Difficult to observe at a walk or in trotting a straight line; consistently apparent under certain circumstances (i.e. weight carrying, circling, inclines, hard surfaces, etc.)
- 3. Consistently observable at a trot under all circumstances.
- 4. Obvious lameness: marked nodding, hitching or shortened stride.
- 5. Minimal weight-bearing in motion and/or at rest;inability to move.

Lameness scores from each viewer were added by testing period and divided by the number of lameness evaluations (6 horses x 2 evaluators) to arrive at the mean (X±SEM) lameness score for that testing period.

## c. Coordinate System

An orthogonal coordinate reference system was established and became an integral part of the calibration structure and the calibrated space used in this project. The origin was defined as being twenty five centimeters below control point 1 (Figure 2, Table 3) at the intersection of the treadmill belt surface and calibration string one. Three mutually orthogonal axes, X,Y,Z, were used to define the reference space and are described below. The positive X-axis of the coordinate system was defined as being coincident with the vector extending from control point 1 to 5 on the calibration structure. The positive Y-axis of the coordinate system was defined as being coincident with the vector extending from control point 1 to 13 and the positive Z-axis of the coordinate system was defined as being coincident with the vector extending from control point 1 to 4 on the calibration structure.

Table 3. X,Y, and Z coordinates of the 16 control point calibration structure used to define the reference coordinate system origin and coordinate directions.

Control Point	X(cm)	Y (cm)	Z (cm)
1	0	0	 25
2	0	0	75
3	0	0	140
4	0	0	190
5	290	0	25
6	290	0	75
7	290	0	140
8	290	0	190
9	290	101	25
10	290	101	75
11	290	101	140
12	290	101	190
13	0	101	25
14	0	101	75
			140
			190
	Point  1 2 3 4 5 6 7 8 9 10 11 12	Point  1 0 2 0 3 0 4 0 5 290 6 290 7 290 8 290 9 290 10 290 11 290 11 290 12 290 13 0 14 0 15 0	Point  1

## d. Calibration Procedure

Calibration provides the necessary coefficients for direct linear transformation (DLT) so the unknown three dimensional positions of subject targets can be determined during subsequent motion. The 16 control point calibration structure (Figure 2) supplied the minimum 6 non-coplanar control points required to perform

**DLT.**<sup>29</sup>

The structure was made of 15 cm diameter PCV piping in a table shape to facilitate its placement and removal from the treadmill. Conventionally, control points should fully encompass the object space where the anticipated motion will occur, so control points on calibration strings were suspended around the perimeter of the structure.<sup>79,82</sup>

The calibration structure was raised to the supporting arms of the treadmill and the volume calibrated each day before data was acquired. The volume contained by the calibration strings was verified using a tape measure. It was measured and adjusted until it contained 290 cm in the X direction, 101 cm in the Y direction and 190 cm in the Z direction as seen in Table 3. Four, 3.5 cm diameter, spheres were suspended by each calibration string at distances of 25, 75, 140 and 190 cm respectively, beginning at the treadmill surface and working in a positive Z direction. The 16 suspended spheres were covered in retro-reflective tape<sup>1</sup> to aid in their identification during data acquisition. The four calibration strings were numbered counterclockwise (Table 3). Six seconds of data were collected in two separate trials and assimilated at a work station using computer software<sup>1</sup> with three-dimensional capabilities. The precision of the testing equipment was routinely validated during the calibration procedure since sixteen

i. No. 7610 High grain sheet, 3M Center, St. Paul, MN.

j. Expert Vision-3D, Motion Analysis Corp., Santa Rosa, CA.

control points were used when only six were needed. The known volume of the calibrated space (5.56 m³) was determined from the product of length x width x height. Actual measured distances between targets on the calibration structure are also estimated by the motion analysis system. The overall difference between these values give a measurement of error known as the norm of residuals. To consistently provide accurate three-dimensional target location values, the calibrated space was adjusted until the norm of residuals were below 5 pixels in this 5.56 m³ volume. Care was taken not to move or jar the cameras once the calibration procedure was complete since any change in camera orientation relative to the initial placement of the calibration structure would make future DLT calculations completely erroneous.

#### e. Target Locations

Retro-reflective targets were fixed to the subjects to improve limb segment and joint identification (Table 4).<sup>85</sup> The targets were made from 3.5 cm spheres covered in retro-reflective tape. Targets were placed over areas with minimal soft tissue covering to limit soft tissue motion and close to the instant center of rotation of each joint to optimize the kinematic information obtained from the metacarpophalangeal joint and the carpal joint complex.<sup>34</sup> Hair was removed from the areas specified in Table 4 to improve target adhesion and to allow placement of targets at identical locations for all video taping sequences. Targets were fixed

to the horses using 5 cm elastic bandage material which encircled the leg at each location. This technique eliminated target without restricting limb movement.

Table 4. Anatomic locations of reflective spheres fixed to trotting subjects during data acquisition.<sup>82</sup>

Target Number	Target Location		
1.	Squamous Part of Occipital Bone (Pole)		
2.	Left Rostral Facial Crest		
3.	Right Rostral Facial Crest		
4.	Dorsal Thoracic Spinous Processes (Withers)		
5.	Left Proximal Lateral Radial Tuberosity		
<b>6</b> .	Left Styloid Process of Distal Radius		
<b>7</b> .	Left Distal Lateral Tuberosity of Metacarpus III		
8.	Left Distal Lateral Hoof		
9.	Right Proximal Lateral Radial Tuberosity		
10.	Right Styloid Process of Distal Radius		
11.	Right Distal Lateral Tuberosity of Metacarpus III		
12.	Right Distal Lateral Hoof		

# f. Data Acquisition and Reduction

Orientation of the treadmill, subjects, videocameras and the object reference coordinate system can be obtained from Figure 2. Once targets were applied, subject coordinates direction were defined similar to the calibration structure. As

horses trotted on the treadmill at 4 m/s, sisty seconds of video data was collected by a hand held video recorder (from V1 and V2) for future subjective lameness evaluation. Quantitative data were acquired by the four symmetrically placed cameras as the subjects trotted for 6 seconds at 4 m/s. The horse was walked at 1.8 m/s while the computer assimilated the first trial of video data, then a second 6 seconds of trotting data was acquired at 4 m/s. Four files of two- dimensional data were processed at a computer workstation to produce one file of three dimensional data for each horse during each trial period.

## h. Data Processing and Variables Assessed

Seven variables were assessed in this project: stance phase, forelimb abduction, stride length, carpal range of motion, fetlock range of motion and withers and head excursion for both sound and lame limbs. During these six second trials, numeric values increased and decreased in a sinusoidal fashion (Table 5), since the variables were measured an average of ten times. Mean values were calculated by summing the differences between maximums and minimums and dividing by the number of cycles during that trial. A sample calculation appears in Table 5. The average values were calculated in an identical manner for all variables evaluated and will be referred to as the average value calculation. These were expressed in degrees (joint angles), centimeters (excursions) or seconds (stance phase).

Table 5. Representative baseline data of computer generated three dimensional poll target coordinates demonstrating the sinusoidal increases and decreases of Z coordinate (Even frames). The formulae used in the average value calculation for head rise coupled to the left (i) forelimb support phase and the right (ii) forelimb support phase appears below.

PATH	FRAME	TIME	x	Y	Z
NUMBER	NUMBER	sec	CIR	CM	CM
1	54	0.883	245.5185	47.8739	166.6692
1	56	0.917	244.9152	47.7814	166.2245 ** a
1	58	0.950	244.8967	47.6546	166.5386
1	60	0.983	245.0040	47.4349	167.5976 Left forelimb
1	62	1.02	244.5557	47.1966	169.1236 in support
1	64	1.05	243.7454	47.0870	170.4760
1	66	1.08	243.2917	46.9300	170.9129 ** b
1	68	1.12	243.5264	46.3890	170.3011
1	70	1.15	243.9352	45.4987	169.0642
1	72	1.18	243.8799	44.5564	167.6629
1	74	1.22	243.4957	43.4358	166.5468
1	76	1.25	243.3143	42.1972	166.1405 ** c
1	78	1.28	243.6108	41.1188	166.6010
1	80	1.32	243.7592	40.6015	167.8391 Right forelimb
1	82	1.35	243.2722	40.7885	169.4671 in support
1	84	1.38	242.7308	41.0920	170.7428
1	86	1.42	242.6822	41.0477	171.0273 ** d
1	88	1.45	243.0912	40.8791	170.7120
1	90	1.48	243.6101	40.9668	169.5092
1	92	1.52	243.8729	41.2753	168.0084
1	94	1.55	244.0097	41.5805	166.8166
1	96	1.58	244.4498	41.6661	166.4483 ** e
1	98	1.62	245.1632	41.7742	167.0822
1	100	1.65	245.4481	42.3674	168.4888 Left forelimb
1	102	1.68	245.2071	43.3480	168.9718 in support
1	104	1.72	245.0560	44.2248	170.0994
1	105	1.73	245.0190	44.5060	170.3645 ** f
1	106	1.75	244.9219	44.6998	170.3215
1	108	1.78	244.2479	44.8850	169.4507
1	110	1.82	242.9728	44.8920	167.9985
1	112	1.85	241.8448	44.7231	166.4141
1	114	1.88	241.3463	44.3363	165.1517
1	116	1.92	241.2020	44.0168	164.7218 ** g
1	118	1.95	240.6676	44.1598	165.3257
1	120	1.98	239.7712	44.4910	165.3257 Right forelimb
1	122	2.02	238.9394	44.8324	166.6844 in support
1	124	2.05	238.0179	45.0480	168.2590
1	126	2.08	237.0815	44.9137	169.3972
i	128	2.12	236.2538	44.9646	169.6518 ** h

i. 
$$[(b-a) + (f-e) + ...]$$
  
 $n^*$ 
ii.  $[(d-c) + (h-g) + ...]$ 

<sup>\*</sup> n equals event frequency.

The sinusoidal progression of numbers produced for carpal range of motion (carpal joint complex), fetlock range of motion and forelimb abduction were derived identically by taking the scalar product between the vectors formed, as defined by target positions. A vector is defined as a directed line in space that had both magnitude and direction, while a scalar product is defined as the smallest angle formed between two vectors. Specific vectors are described below for the left limb only. To determine the range of motion of the carpal joint complex in degrees, a vector was constructed from the proximal to distal extremity of the radius (from target 5 to target 6). A second vector was constructed from the distal extremity of the radius to the distal extremity of the metacarpus (from target 6 to target 7). Once these vectors were formed the scalar product was determined. Using the average value calculation, the average maximum flexion that occurred on the caudal aspect of the carpus during that trial period was determined.

To determine the range of motion of the fetlock joint in degrees, a vector was constructed from the distal extremity of the radius to the distal extremity of the metacarpus (target 6 to target 7). A second vector was constructed from the distal extremity of the metacarpus to the distal aspect of the hoof (target 7 to target 8). Next, the scalar product and average value were calculated to determine the average maximum flexion that occurred on the palmar aspect of the fetlock joint during that trial period.

To determine forelimb abduction in degrees, a vector was constructed from the proximal to distal extremity of the radius (target 5 to target 6). Next a scalar product was taken between this vector and the Y axis of the reference coordinate system. Mean value calculation provided the average maximum abduction that occurred in both forelimbs during that trial period.

Mean values for head and withers excursion were determined from the sinusoidal progression of targets 1 and 4 respectively, in the Z axis. Head and withers excursion were coupled with the left and right forelimbs so that excursion average values represented the average distance, in cm, the head or withers targets travelled in the positive Z axis while the sound or lame leg was in its support phase.

Stance phase was defined as the time period where there was no vertical displacement of the hoof (target 8). In other words, it was the time associated with the Z value of the hoof target when it was between 0 to 3.5 cm in height. Using the average value calculation, this provided a number which represents the average time the hoof remained in contact with the ground.

Calculation of stride length involved the sinusoidal progression of hoof target X trajectories (target 8), using three-dimensional position data. This provided a value representing the average distance, in cm, the hoof travelled in a caudal to cranial direction for that time period. The average value was determined as described

above.

# D. Statistical Analysis

The effects of intra-articular amphotericin-B on stride length, stance phase, forelimb abduction, carpal and fetlock range of motion and head and withers excursion for the sound and the lame limb were analyzed using as two way analysis of variance ( $P \le 0.05$ ). The Student-Newman-Keuls test was used to evaluate differences between means.<sup>86</sup> The subjective effect of amphotericin-B on lameness was analyzed using the Friedman's Block Test ( $P \le 0.05$ ). The Signed Ranks test was used to evaluate differences between means.<sup>86</sup>

# **VI.RESULTS**

The average subjective evaluation of forelimb lameness was graded 0/5 (clinically sound horses) during baseline measurements. The mean  $(X\pm SEM)$  subjective lameness grade was significantly increased during all measurement periods when compared to baseline values (Figure 3).

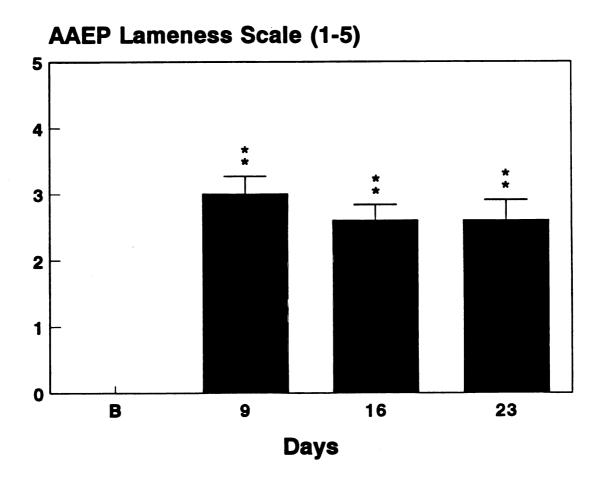


Figure 3. The mean (X $\pm$ SEM) subjective lameness scores as determined by two independent examiners during baseline measurements (B) and 9,16 and 23 days post induction of arthritis. Examiners used the AAEP lameness classification scale.<sup>84</sup> (\*\*=P $\leq$ 0.01)

The pattern of head movement was affected by lameness induction. During baseline measurements, the head consistently moved in a symmetric sinusoidal pattern (Horse 1, Figure 4a). The head target was raised and lowered during the support phase of each forelimb, producing an up and down head movement for the support phase of each forelimb. There was no significant difference in head excursion when either forelimb was in its support phase (Figure 11).

However, when the subjective lameness grade was 3/5, the head moved in a sinusoidal pattern, but lacked symmetry (horse 4, Figure 4b). Similar to baseline measurements, the head target was raised and lowered during the support phase of each forelimb, but the amount of head excursion that occurred while the lame limb was in its support phase was decreased significantly (Figure 11).

When the subjective lameness was 4/5, the upward head excursion that occurred while the lame leg was in its support phase was completely eliminated (horse 2, Figure 4c). The head was at its maximum height just prior to the support phase of the lame limb and dropped throughout the support phase. Then the head was elevated to its maximum height during the support phase of the sound limb before weight bearing occurred on the lame limb. This pattern reduced the frequency of head excursion by 50 percent in the lamest horses (Figure 4a vs 4c). The pattern of withers target movement mimicked the head target both during baseline measurements and after the induction of lameness.

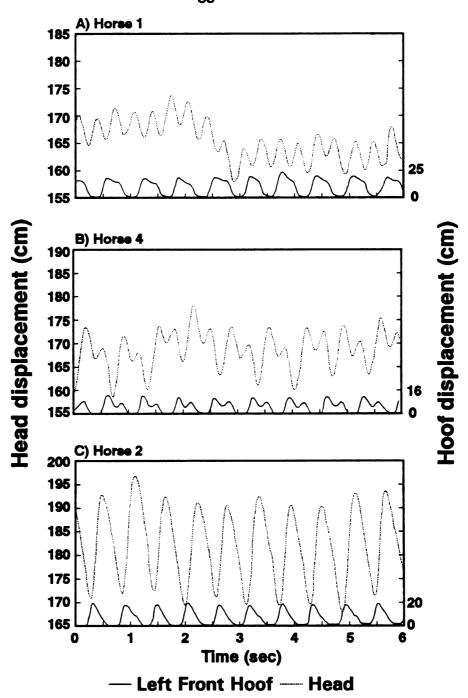


Figure 4. Representative verticle displacement (Z component in cm) of the head (target 1) and left front hoof (target 8) as horses trotted (4 m/s for 6 seconds) on the treadmill. a) Recording of head movement before arthritis induced (horse 1). b) Recording of head movements post arthritis induction for a 3/5 subjective lameness grade (horse 4). c) Recording of head movements post arthritis induction for a 4/5 subjective lameness grade (horse 2).

The mean (X  $\pm$  SEM) values for all variables measured for the sound and lame limb are listed in Table 6.

Table 6. The effect of amphotericin-B induced arthritis on stride length, stance phase, carpal and fetlock range of motion, forelimb abduction, head and withers excursion are listed in the following order: the sound limb then the lame limb. All values are presented as means  $(X\pm SEM)$ . (#=range of motion, \*=p<0.05, \*\*=p<0.01)

## MEASUREMENT PERIOD (Days)

Variable	В	9	16	23
Stride	99.64 <u>+</u> 1.6	102.71 <u>+</u> 1.7	99.80 <u>+</u> 1.1	100.51 <u>+</u> 2.1
Length (cm)	98.54 <u>+</u> 1.5	93.91 <u>+</u> 3.8	95.63 <u>+</u> 3.3	97.37 <u>+</u> 3.0
Stance	0.22 <u>+</u> 0.01	0.22 <u>+</u> 0.03	0.22 <u>+</u> 0.00	0.22 <u>+</u> 0.01
Phase (sec)	0.21 <u>+</u> 0.01	0.20 <u>+</u> 0.01	0.21 <u>+</u> 0.01	0.22 <u>+</u> 0.01
Forelimb	8.32 <u>+</u> 1.6	7. <del>9</del> 8 <u>+</u> 1.1	7.75 <u>+</u> 1.4	8.54 <u>+</u> 1.4
Abduction (degrees)	8.32 <u>+</u> 1.0	7.97 <u>+</u> 0.7	9.15 <u>+</u> 1.0	8.86 <u>+</u> 1.4
Carpal	66.94 <u>+</u> 4.3	58.06 <u>+</u> 2.5	62.80 <u>+</u> 2.4	61.79 <u>+</u> 2.9
R.O.M.* (degrees)	68.10 <u>+</u> 3.8	63.07 <u>+</u> 1.9	62.50 <u>+</u> 2.7	65.40 <u>+</u> 3.1
Fetlock	87.41 <u>+</u> 2.6	88.96 <u>+</u> 3.1	88.01 <u>+</u> 2.8	88.09 <u>+</u> 2.7
R.O.M.* (degrees)	86.24 <u>+</u> 2.2	72.95 <u>+</u> 4.5 <sup>**</sup>	75.11 <u>+</u> 3.2°	76.81 <u>+</u> 4.9*
Head	4.82 <u>+</u> 0.5	12.79 <u>+</u> 2.2°	11.12 <u>+</u> 2.1 <sup></sup>	10.91 <u>+</u> 2.4"
Excursion (cm)	4.57 <u>+</u> 0.32	1.80 <u>+</u> 0.83**	2.16 <u>+</u> 1.00**	2.31 <u>+</u> 0.87**
Withers	5.13 <u>+</u> 0.2	7.38 <u>+</u> 0.7*	6.93 <u>+</u> 0.7	6.95 <u>+</u> 0.7*
Excursion (cm)	4.77 <u>+</u> 0.4	2.55 <u>+</u> 0.9	3.15 <u>+</u> 0.8	3.67 <u>+</u> 0.8

The stance phase of the right and left forelimbs was  $0.22\pm0.01$  and  $0.21\pm0.01$  seconds respectively (X $\pm$ SEM), with horses trotting at 4m/s on a treadmill. Lameness induction did not affect stance phase (Figure 5).

Forelimb abduction of the right and left forelimb was  $8.32 \pm 1.6$  and  $8.32 \pm 1.0$  degrees respectively. The intra-articular injection of amphotericin-B did not significantly affect forelimb abduction (Figure 6).

Baseline measurements of stride length for the right and left forelimbs was  $99.64\pm1.6$  and  $98.54\pm1.5$ , cm respectively (X $\pm$ SEM) as horses trotted on the treadmill. Stride length was unaltered by lameness induction (Figure 7).

Carpal range of motion of the right and left forelimbs was 66.94±4.3 and 68.10±3.8 degrees respectively, as horses trotted at 4 m/s. Carpal range of motion was decreased significantly nine days after the induction of arthritis in the sound limb (Figure 8), but returned toward baseline values by day 16 after arthritis induction.

Baseline measurements of fetlock range of motion for the sound and lame forelimbs was  $87.41\pm2.6$  and  $86.24\pm2.2$  degrees respectively. Although fetlock range of motion of the sound limb was not affected significantly by the intra-carpal injection of amphotericin-B, it was decreased significantly during all measurement periods in the lame forelimb (Figure 9).

Withers excursion of the right and left forelimbs was  $5.13\pm0.2$  and  $4.77\pm0.4$ 

cm respectively. Withers excursion was increased significantly during all lameness measurement periods while the sound limb was in its support phase, and decreased significantly only at day nine while the lame limb was in its support phase (Figure 10).

Baseline measurements of head excursion for the right and left forelimb was  $4.82\pm0.5$  and  $4.57\pm0.32$  cm respectively, while horses trotted on the treadmill at 4 m/s. After lameness induction, head excursion was increased significantly compared to baseline values during all measurement periods when the sound forelimb was in its support phase and decreased significantly when the lame forelimb was in its support phase (Figure 11).

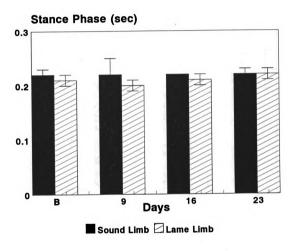


Figure 5. Stance phase (X±SEM in seconds) of the sound forelimb and the lame forelimb before (B) and 9, 16 and 23 days after lameness induction.

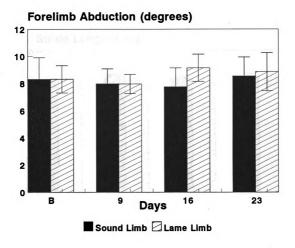


Figure 6. Forelimb abduction ( $X\pm$ SEM in degrees) of the sound forelimb and the lame forelimb before (B) and 9, 16 and 23 days after lameness induction.

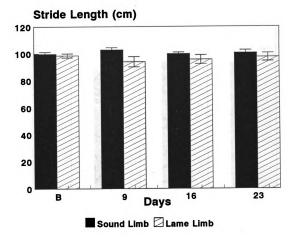


Figure 7. Stride length ( $X\pm SEM$  in cm) of the sound forelimb and the lame forelimb before (B) and 9, 16 and 23 days after lameness induction.

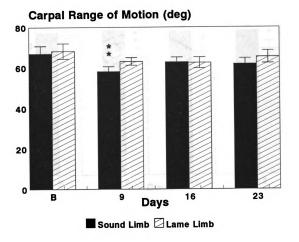


Figure 8. Carpal range of motion (X $\pm$ SEM in degrees) of the sound forelimb and the lame forelimb before (B) and 9, 16 and 23 days after lameness induction. (\*\*=p $\leq$ 0.01)

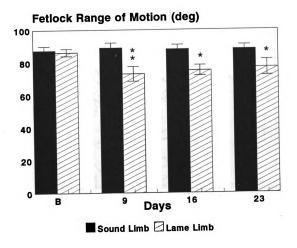


Figure 9. Fetlock range of motion (X $\pm$ SEM in degrees) of the sound forelimb and the lame forelimb before (B) and 9, 16 and 23 days after lameness induction. (\*=p $\le$  0.05,\*\*=p $\le$ 0.01)

# Withers Excursion (cm)

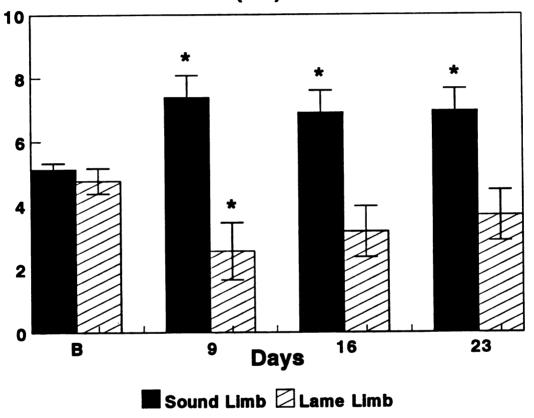


Figure 10. Withers excursion (X $\pm$ SEM in cm) initiated during the support phase of the sound forelimb and the lame forelimb before (B) and 9, 16 and 23 days after lameness induction. (\* = p $\le$ 0.05)

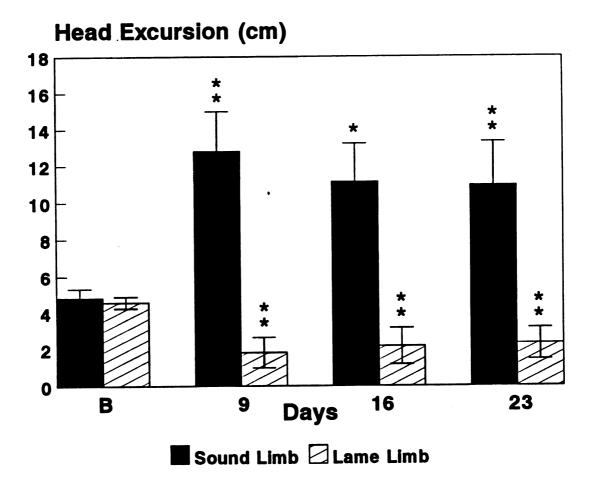


Figure 11. Head excursion (X $\pm$ SEM in cm) initiated during the support phase of the sound forelimb and the lame forelimb before (B) and 9,16 and 23 days after lameness induction. (\* =p $\leq$ 0.05 \*\*=p $\leq$ 0.01.)

### VII. DISCUSSION

The computer-assisted three-dimensional video gait analysis technology used in human medicine were successfully adapted for locomotion analysis in the horse. Amphotericin-B was used to induce a unilateral forelimb lameness of two weeks duration and alterations in the equine trotting gait were determined and compared to baseline values.

Head movements in the sound and lame horse were determined to be the most sensitive indicators of lameness (Figure 11). Similar to a sound horse at a walk <sup>87</sup>, our horses heads fell and rose in a sinusoidal fashion during the support phase of each forelimb (Figure 4) while trotting and this fall-rise frequency was reduced by up to half in the lame horse. In contrast to previous reports, <sup>6,53,54</sup> the head fell during the lame forelimb support phase and rose when the sound diagonal pair was on the ground.

Withers movements were determined to be the next most sensitive indicator of lameness. Withers movement closely paralleled head movement, as previously speculated, <sup>88</sup> in both the sound and lame trotting horse.

Several theories exist regarding the asymmetric movement of the head and neck in the lame horse. These include an attempt to reduce the weight supported by the affected limb,<sup>54</sup> and an effort to shift the center of gravity caudally, away from the lame forelimb, as the head is elevated.<sup>6</sup> An alternate theory to explain the

head and neck movements seen in unilateral forelimb lameness might be an uncoupling of their weight from the lame forelimb as the head and neck experience a "free fall" like phenomenon. This logically follows, knowing that the head and neck represent 10 % of body weight,<sup>51</sup> and that the head and withers were lowered during the support phase of the lame limb in this project. This theory is supported by three different quantitative studies which examine the kinetics of unilateral forelimb lameness using force platforms. 8,55,56 In these articles, it was uniformly demonstrated that the injured forelimb of walking or trotting horses have significantly decreased vertical forces and cranio-caudal decelerative horizontal forces. It was also noticed that horizontal decelerative forces are increased and horizontal accelerative forces are decreased in the sound forelimb.<sup>8,55,56</sup> This theory is also supported by two publications demonstrating that the hip drops during the support phase of the lame hindlimb. 87,89 In this project, withers excursion decreased when the lame forelimb was on the ground limb, and movements of the withers and head occurred together. It would seem logical that the hip and withers would move in a similar manner to compensate for lameness. therefore providing further support to the "free fall" theory.

Stance phase, defined as the time the limb is in contact with the ground,<sup>50</sup> averaged around 220 msec for both the sound and lame limb and was not significantly affected by the induction of lameness. Similar stance phase times of

242 msec were demonstrated in an induced carpal lameness model where a force platform analysis was used in horses trotting between 2.7 and 2.9 m/s.<sup>8</sup> In contrast, published case reports have shown stance phase to both increase<sup>47</sup> and decrease with lameness.<sup>48,49</sup>

Though quantitative reports describing the lame horse are uncommon, it has been demonstrated in 30 sound trotting standardbreds using high-speed cinematography, that variations in limb movement in any particular horse were very small.45 It is theorized that the symmetric nature of animal gait will allow individual asymmetries to be identified as lameness. It has been suggested that a lame horse may alter the swing phase of the lame limb to minimize the distressing aspects of the lameness.<sup>6</sup> and therefore affect stride length. Stride length is defined by convention as the horizontal distance covered in the plane of progression beginning with the stance phase of one limb and ending with the subsequent placement of that same limb on the ground. 9,50 To simplify calculations in this project, stride length was re-defined as the excursion one limb makes in the positive X direction, because this value would be exactly half of that determined conventionally. It is conceivable that the definition for stride length may change, if future lameness evaluation studies are standardized by using a treadmill. In our study stride length was not significantly altered by the induction of lameness. Stashak suggests<sup>54</sup> that changes in the cranial and caudal phases of stride are the important issue since the length of stride of the affected limb must remain the same as the contralateral limb if the horse is to travel in a straight line. A horse with a decreased cranial phase of stride may compensate by increasing the caudal phase of stride in the same limb and therefore leave the stride length unaffected. This may have occurred in this project and could be particularly true on a treadmill but these aspects were not examined. Stride frequency, defined as the time required to complete one stride, 50 did not change with the induction of lameness, since stride length was unaffected and treadmill speed was held constant at 4 m/s.

Forelimb abduction and carpal range of motion were evaluated because it has been hypothesized that carpal lameness causes a horse to move with a paddling or circumducted gait due to the pain flexion induces. However, in this study carpal range of motion and forelimb abduction were not consistently significantly affected in either the sound or lame forelimb during any of the measurement periods. These findings may have validity only for unilateral forelimb lameness whereas, the paddling gait may occur with bilateral forelimb lameness.

Fetlock range of motion in the sound limb was not altered by unilateral forelimb lameness, but a significant decrease in range of motion occurred in the lame limb during all lameness measurement periods (Figure 9). It is theorized,<sup>54,88</sup> and has been demonstrated in 25 horses at a walk using a force platform, that force

compensation is provided by the diagonal hind limb in a unilateal forelimb lameness, since these limbs are in their support phase at the same time.<sup>91</sup> Though right diagonal hind limb movement was not examined in this project, this diagonal hind limb compensation could explain a normal fetlock range of motion in the sound limb and significant changes in the lame forelimb fetlock range of motion.

Amphotericin-B was used to induce arthritis in this project because, although the inciting cause for DJD is unknown, primary soft tissue inflammation is one of the proposed theories in horses and has been implicated as the primary cause of osteoarthritis in man.<sup>58</sup> Additionally, synovitis is almost always present in cases of equine degenerative joint disease<sup>59,60</sup> and the polyene antibiotics<sup>73,74</sup>, specifically amphotericin-b<sup>73</sup> have been used successfully to create synovitis in the horse. Using the dosages described in this project, it provided a synovial arthritis and a consistent lameness of 2 weeks duration.

Lameness has been defined as a deviation from the normal gait due to pain or mechanical dysfunction.<sup>84</sup> One of the deleterious side effects of experimental arthropathies is discomfort and pain. Pain, defined as the activation of a discrete set of receptors and neural pathways, are usually aroused by stimuli that are noxious or damaging to tissues.<sup>92</sup> The sensitivity of a tissue to pain perception is directly related to the presence of nociceptors, or pain receptors.<sup>93</sup> Nociceptors

are numerous in the periosteum, joint capsule and tendons. The prostaglandins released during tissue damage are known to enhance the sensitivity of nociceptors to pain while cyclo-oxygenase inhibitors, like phenylbutazone, provide analgesia by inducing a pronounced suppression of the inflammatory response. When nociceptor are stimulated by a chemical insult, a nerve impulse is produced and conducted along the afferent fibers. These afferent fibers enter the spinal cord from the dorsal root and terminate on dorsal horn neurons. The dorsal horn contains neurons that can project the nociceptive message to higher brain centers, relay the message to other cells and inhibitory interneurons that modify the nociceptive message. Pain modulation can therefore occur at the nociceptor, the dorsal root or at higher brain centers.

Phenylbutazone was used once in the protocol as a single oral dose to modulate the acute inflammatory reaction. It remains a widely used and effective non-steroidal anti-inflammatory drug (NSAID) for the equine species, <sup>95</sup> because its acidic nature potentiates drug accumulation in inflamed tissues. <sup>96,97</sup> Synovitis is often reduced by a brief regimen since synovial fluid levels approach 50% of the plasma concentration. <sup>96</sup> Phenylbutazone can cause an irreversible antagonism of the cyclo-oxygenase enzyme for as long as 12 to 24 hours, reduce tissue prostaglandin (PG) production and thus prevent the sensitization of pain receptors. <sup>97,99</sup> In this project, analgesia was not provided exclusively at the

receptor by using phenylbutazone, because our protocol required a synovitis of two weeks duration.

Opioids activate opioid receptors (agonist), block opioid receptors (antagonist) or do either (agonist-antagonist) depending on the site they stimulate. 100 Identified opioid receptors include the mu, kappa, sigma and delta receptors. 100 It is known that opioid analgesics interact at selective endogenous recognition sites and that a high opioid receptor density exists in the dorsal horn of the spinal cord and certain subcortical regions of the brain.94 The opioid butorphanol tartrate was used as the primary analgesic in the protocol since opioids can inhibit pain transmission from primary afferent nociceptors, modulating neurons or directly affect pain transmission in the spinal cord, brain stem and various parts of the brain.99 Butorphanol tartrate, a synthetic morphinan that is chemically related to naloxone, 101 acts as a competitive antagonist at the mu receptor and an agonist at the sigma and kappa receptor. It specifically has a high affinity for kappa receptors which are believed to be of primary importance in analgesia. 102 It is an effective analgesic, as has been demonstrated in several animal pain model studies 103-105, and was noted to provide analgesia for cancer pain, post surgery pain, post surgical cancer pain and prepartum pain. 108 Butorphanol was used to provide analgesia in this project because it has a rapid onset of action, is centrally acting so it does not interfere with the inflammatory process of this synovitis model and is efficacious when given intramuscularly.<sup>102,103</sup> Also, it was easy to use in a clinical setting since it is not a scheduled drug<sup>103</sup> and has relatively few deleterious effects.<sup>99</sup>

The fourth edition of the 1991 American Association of Equine Practitioners (AAEP) Guide for Judging of Equestrian Events contains a subjective lameness classification scale.84 This classification scheme is a commonly used lameness scale and was used in this project to provide continuity with current practices, to determine the subjective degree of lameness produced by this model, to ascertain how subjective and objective lameness evaluation compared and to provide an example of the arbitrary scales available for lameness evaluation. Both computer determinations and subjective evaluation of the vertical component of head movements demonstrated a significant change when compared to baseline values. In contrast, subjective evaluation perceived head fall to occur while the sound forelimb was weight bearing and objective evaluation demonstrated head fall to occur while the lame forelimb was in its support phase. Since subjective evaluation has been proven unreliable, 32,33 measurement systems which generate quantitative information are preferred 9 over visual assessment techniques.

Target motion is always an issue when used to improve limb and body segment identification since these targets are prone to measurement errors due to skin movement. 9,26,34,35. There are concerns that target motion may limit the usefulness

of cinematography and videography in assessing joint angles. 26,34,107 since all automated motion analysis systems require the attachment of markers to the skin. The body locations selected for targets positions (Table 4) were bony landmarks with minimal soft tissue or muscle coverage to minimize this effect. 34,35,37 Target motion errors have been quantified in 6 normal horses at a walk.<sup>35</sup> demonstrated that target motion perpendicular to the long axis of the limb was undetectable at all sites measured and that substantial motion of targets parallel to the long axis of the limb only occurred proximal to the carpus and tarsus. It is likely that changes in skin marker position are more significant at higher speeds<sup>34</sup> and around joints proximal to the carpus, 26,35 therefore our concerns for target movement were restricted to the proximal lateral radius (targets 5 and 9, Table 4). Assuming that quantified target movement occurred only in a proximal distal direction and not a cranio-caudal direction, 35 it was inferred that target movement in this project was in a direction co-linear with the proximal vector and did not significantly underestimate the calculated angles formed in the carpal joint complex.

The goal of studying equine joint disease and performing quantitative lameness evaluation using experimentally induced arthritis is identifying precise diagnostic criteria for lameness so the site of pain or mechanical dysfunction could be determined in a clinical setting.<sup>12</sup> It is recognized, however, that amphotericin-B

and other experimental models do not completely reproduce the clinical and pathological changes seen in naturally occurring equine DJD and are limitations in the experimental design.<sup>73,74</sup>

Treadmill evaluations have been used extensively for upper respiratory tract evaluations and, when combined with a videoendoscopic exam, has proven to be a very safe and useful diagnostic procedure. Fredricson et al have demonstrated the gait reproducability between exercising sessions to be good, and found a good correlation between the gait repeatability on the track and on the treadmill. The treadmill allowed kinematic analysis to occur in a controlled environment, enabled horses to be assessed indoors under standardized conditions and form of exercise. The central location of a treadmill facilitated the positioning of multiple cameras which allowed three-dimensional analysis to be performed on multiple gait cycles. Hoof visibility was not complicated, as seen on dirt and track surfaces, due to the less compliant rubber-steel make-up of the belt. The treadmill proved to be a safe and useful device for lameness evaluation.

## VII. SUMMARY AND CONCLUSIONS

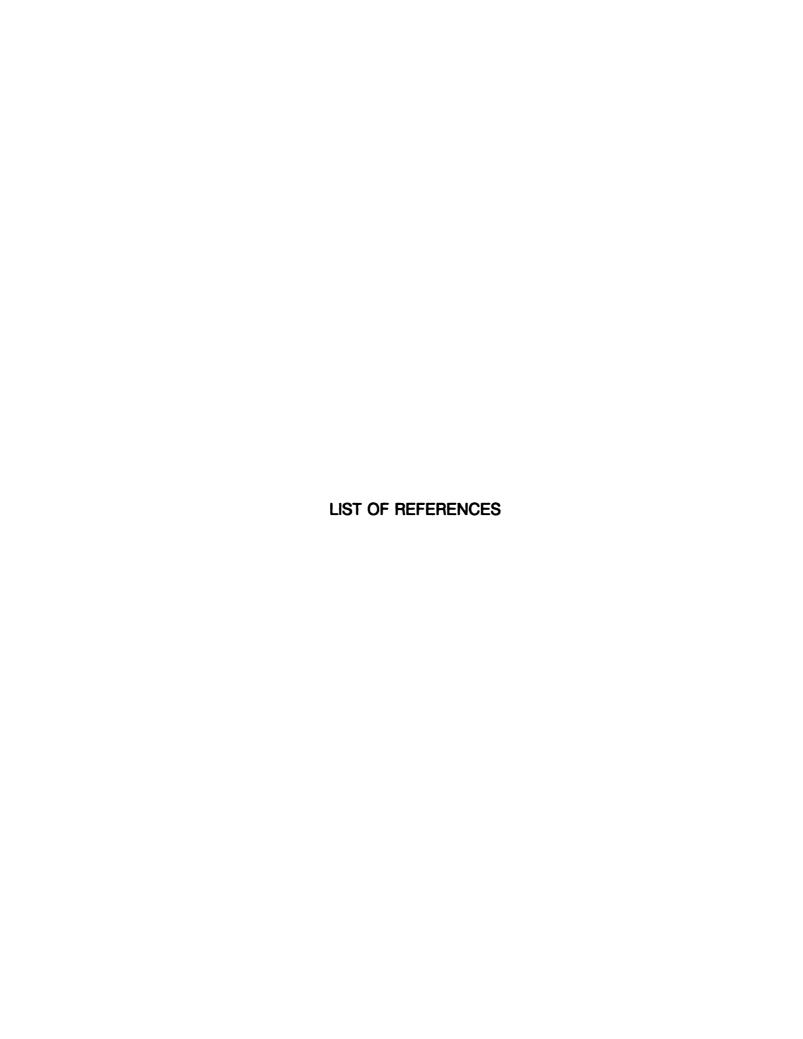
It has been stated that the complex movement of quadrupeds requires skilled analysis at many levels and that the generated data must be reduced to a simpler form if rational decisions are to be formed regarding clinical cases.<sup>6,14</sup> Using

amphotericin-B to create a unilateral forelimb lameness and a computer work station with three dimensional software capabilities, the following conclusions were drawn.

- 1. The computer-assisted video gait analysis technology used in this project was successfully adapted from a human laboratory for use in normal and lame horses.
- 2. In normal horses, the vertical component of head and withers movement occurred synchronously and symmetrically in a sinusoidal fashion, during the support phase of both forelimbs. Using this forelimb lameness model, the head and withers continued to move synchronously, but the symmetry between sound and lame forelimbs was no longer present. Head and withers motion, determined to be the most sensitive variables, were noted to drop during the support phase of the lame forelimb and rise during the support phase of the sound forelimb.
- 3. The baseline measurements for forelimb abduction and stance phase for the sound forelimb, the lame forelimb and the sound to lame forelimb ratio were not significantly different from any of the measurement periods. These variables were concluded to be unreliable for the assessment of equine carpal lameness.
- 4. Measurement of stride length was significantly different from baseline values when assessing the sound to lame forelimb ratio at day nine only. Carpal range of motion was significantly different from baseline values when assessing the

sound forelimb at day nine only. These variables were concluded to be unreliable for the assessment of equine carpal lameness.

5. Fetlock range of motion in the lame forelimb and sound to lame forelimb ratio; withers excursion when the lame limb is in its support phase and head excursion for the sound, lame and the sound to lame forelimb ratio were significantly different from baseline values during all measurement periods and were concluded to be reliable in the assessment of equine carpal lameness.



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