The microvasculature in the equine distal phalanx: Implications for fracture healing

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Summary
Objectives: To describe the intra-osseous microvasculature of the distal phalanx of the equine forelimb with regard to its potential clinical relevance.

Methods: Eleven clinically normal equine forelimbs were used from six adult horses (range: 4 to 18 years old) euthanatized for reasons unrelated to lameness. In each limb the median artery was catheterized at the level of the carpus and India ink was injected under constant manual pressure. The limbs were frozen and 5 mm thick sections of the foot were cut in the sagittal, coronal, or transverse planes on a band saw. The sections were fixed in 10% formalin and cleared, the sections were photographed and using a modified Spalteholz technique. Once cleared, the sections were photographed and the microvascular anatomy identified.

Results: The vascular injections revealed a rich intra-osseous microvascular supply of the distal phalanx originating from the medial and lateral palmar digital arteries. In addition, numerous smaller vessels from the terminal arch, formed by anastomosis of the medial and lateral palmar digital arteries, could be seen branching into the distal aspects of the distal phalanx. This distal portion of the distal phalanx appeared more densely vascularized than the proximal part in all specimens examined.

Clinical significance: The increased vascularity demonstrated in the distal portion of the distal phalanx appears to correlate with improved fracture healing reported in this area. This may also explain why healing fractures involve both the distal and proximal portions of the distal phalanx have been described as progressing from distal-to-proximal.

Introduction
Fractures of the distal phalanx are most common in racehorses but can occur in horses of all disciplines (1, 2). A significant portion of horses with these fractures never return to their previous level of performance due to incomplete healing (3). These fractures occur predominantly in the forelimbs and are classified into seven different types based on their location (Figure 1) (2, 4-6). The prognosis for horses with each of these phalangeal fractures returning to soundness varies in different regions of the distal phalanx (3, 6). Fractures of the solar margin (Type VI) have an excellent prognosis while fractures through the mid-sagittal body of the distal phalanx (Type III) or extensor process (Type IV) have a guarded to fair prognosis (3, 6). In addition, distal phalangeal fractures involving both the body and solar margin were found to always heal in a distal-to-proximal direction (3, 7).

One predominant explanation for this regional variation cites the joint involvement or the lack thereof. Non-articular fractures (Types I, VI and VII) show greater incidence of complete osseous union than articular fractures (Types II, III and IV) (4). It has been proposed that this may be due to the middle phalanx causing movement of the fracture during weight bearing, or the disruption of the articular cartilage and subsequent filling of the fracture with synovial fluid, forcing the two fragments apart (4). However, this hypothesis does not explain the differences in fracture prognoses among the different types of articular fractures.

Another plausible explanation is that there is regional variation in the microvascular anatomy of the distal phalanx. In the event of a fracture causing vascular injury, an area supplied with few blood vessels is more likely to become ischaemic, and thus have hindered fracture healing (8). While the major extra-osseous and intra-osseous vascular supply has been previously described, the detailed microvascular anatomy of the equine distal phalanx has not been thoroughly examined (9–12). Regional variation in the microvasculature of the equine distal phalanx may explain the differences observed in the healing of fractures occurring in specific locations of the distal phalanx (3, 6).

Therefore, the purpose of the current study was to detail the microvascular
supply to the distal phalanx of the equine forelimb. It was hypothesized that the distribution of the microvasculature of the distal phalanx could provide an explanation for the differential healing reported in these areas and the observed healing of the distal phalanx in the distal-to-proximal direction.

Materials and methods

Collection of specimens

Eleven forelimbs were harvested from six adult horses that were euthanatized for reasons unrelated to forelimb lameness. The horses were four to 18 years of age and the breeds included Arabian (n = 1), Paint (n = 2), Quarter Horse (n = 2), and Thoroughbred (n = 1).

Injection of contrast material

All limbs were disarticulated at the distal radius and the median artery was isolated. A polyethylene catheter was then inserted into the vessel and secured with ligatures of 3.5-metric silk. All visible veins were also ligated with 3.5-metric silk to prevent leakage of the contrast material. The cannulated arteries were then injected with 120–180 ml of India ink\(^a\) using constant manual pressure. To confirm that the vasculature was completely filled down to the capillary level, the skin just proximal to the hoof wall (at the coronary band) was incised and extrusion of ink from the dermal capillaries was verified.

While several other contrast materials (i.e. plastics, resins, barium) and techniques (i.e. casting and microradiography) have been used to demonstrate microvascular anatomy in bone and soft tissues, the degree of vascular penetration into the arterial tree (i.e. arterioles or capillaries) is dependent on the dilution of the injection solution, the suspended particle size, or a combination of both (13–17). The carbon particles in India ink are approximately 40 nm in diameter allowing them to easily pass into and stain capillary vessels (13, 18).

\(^a\) Higgins Waterproof Black India Ink, Sanford, Bellwood, IL, USA

Tissue clearing

Sections underwent processing using the modified Spalteholz technique (19). Briefly, the sections were decalcified in 10% nitric acid and dehydrated in increasing percentages of ethanol. The sections were then defatted in chloroform and cleared using a Spalteholz solution (3 parts benzyl benzoate and 5 parts methyl salicylate). The entire clearing process took approximately three weeks. All sections were then photographed and sections from similar locations were then compared to determine relative vascularity in a specific region.

Results

Vascular anatomy

The major vessels supplying the distal phalanx of the equine forelimb are the medial and lateral palmar digital arteries, which enter the solar foramina of the distal phalanx (Figure 2). Terminal branches of the palmar digital arteries traverse cranially, axially, and slightly dorsally to form mirror-image apexes pointing toward the mid-sagittal line (Figure 3). From these points, the two vessels move axially to anastomose at the midsagittal line, forming a semi-circular vessel called the terminal arch (Figure 3).

From the terminal arch, several prominent rami can be seen extending in both the dorsal (ramus dorsalis arcus terminalis)
and distal (ramus palmaris arcus terminalis) directions (Figures 4, 5, and 6). These vessels, along with terminal branches of the medial and lateral dorsal arteries of the palmar digital arteries, provide a rich microvascular supply to the entire solar margin of the distal phalanx (Figure 2, Figure 4).

Sections of the distal phalanx demonstrated a comparatively limited microvascular supply to the proximal body and extensor process when compared to the solar margin regions (Figure 6, Figure 7). There was no evidence of any additional extra-osseous vascular supply to the extensor process and proximal mid-body of the distal phalanx beyond that provided by smaller branches of the ramus dorsalis arcus terminalis. This was found to be minimal in all specimens examined.

The microvasculature patterns within the distal phalanx appeared qualitatively similar in all specimens and there was no apparent effect of breed or age on these microvascular patterns.

**Discussion**

The major vascular supply to the distal phalanx of the equine forelimb demonstrated in the current study originates from the medial and lateral palmar digital arteries and confirms the observations of earlier studies (8–10). However, the results of the current study also demonstrate that the distal phalanx has a microvascular supply that varies regionally. The greatest concentration of vessels was found throughout the solar margin region while the extensor process and midsagittal region of the mid and proximal body of the distal phalanx demonstrated a comparative decrease in microvasculature. The variation in the extent of microvascularity demonstrated in different regions of the distal phalanx appears to coincide with the regional differences reported in fracture healing in these same areas (2–4, 6, 9, 20, 21). The greatest density of microvasculature was observed in the solar margin region of the distal phalanx. Fractures in this area (Type VI) have been reported to have the best prognosis (6). However, the extensor process and midsagittal region of the mid and proximal body of the distal phalanx showed marked hypovascularity when compared with the rest of the bone. Fractures occurring in these two regions (Types III and IV, respectively) were reported to have the worst prognosis, and often resulted in delayed or non-union of the bone (6).
The correlation between fracture healing and the degree of vascular damage following fracture has been well-established in humans and animals and may explain the relationship between the vascular anatomy described in the current study and the regional variation in healing reported in clinical studies (1, 3, 4, 13, 22–24). Type III fractures, which run through the midsagittal area from the distal interphalangeal joint to the solar margin, probably rupture the terminal arch of the medial and lateral (proper) palmar digital arteries. Because there appears to be a limited amount of collateral circulation in this area, these fractures may significantly compromise the vascular supply in these areas leading to hindered fracture healing. In contrast, solar margin fractures (Type VI) occur in an area of rich microvascular supply which would theoretically allow for much greater blood perfusion in that area. The substantial microvascular supply found in the solar margin region of the distal phalanx and relative hypovascularity of the proximal regions of the distal phalanx may also explain the distal to proximal progression of healing observed in Types I–III and Type VI fractures (7).

A potential limitation of this study was the variety of breeds and ages of the animals from which the anatomic specimens were collected, as well as the limited number of specimens. However, there was marked similarity in the microvascular pattern between all specimens suggesting anatomic consistency across breeds and ages. This absence of any apparent age-related effect on the vascular patterns of bone in healthy adult individuals has also been reported in other species (25–30). Another potential limitation of this study was that the technique of documenting the microvascular anatomy of the equine distal phalanx used in this investigation was qualitative and only reflected the static, structural components of the vascular supply and not the physiological parameters of blood flow and perfusion. However, previous studies have shown an excellent correlation between the qualitative microvascular anatomy of bone and vascular response to acute injury (14).

The results of this study support the hypothesis of an anatomic microvascular rationale for the regional differences in fracture healing reported for the distal phalanx. However, it is also important to recognize that other factors besides vascular supply can contribute to delayed fracture healing. For example, the poorer prognosis for fractures of the extensor process (Type IV) and midsagittal proximal body regions of the distal phalanx (Type III) could also be due to the greater movements or higher local loading forces experienced in these regions secondary to traction of the extensor process, or a combination of both, or hoof wall loading (31–33). Such motions could also compromise fracture healing. In addition, technical factors such as degree of immobilization and degree of anatomic reduction can also affect fracture healing. However, in a healthy individual, if these technical issues are properly addressed, the relative blood supply in the area of the fracture has been shown to have a major influence on whether or not the fracture heals (14). Additional studies examining the sequence of biological events that follow each fracture type in the distal phalanx are needed to identify the precise factors responsible for the regional variations in outcomes.

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Conflict of interest

No conflicts of interest have been declared.

References