

# LOCALIZATION OF p63 TO BASAL EPITHELIAL CELLS OF THE EQUINE (*Equus caballus*) HOOF EPIDERMAL LAMELLAE.\*

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## INTRODUCTION

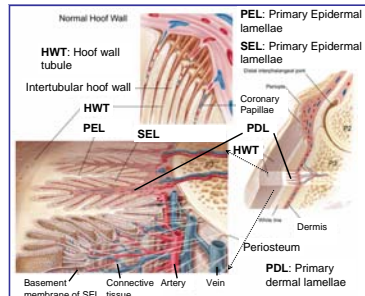
- Equine laminitis is a common, painful, and often fatal condition of horses.
- Laminitis results in destruction of the attachment between epidermal lamellar structures of the hoof and the underlying dermal lamellae and skeletal structures resulting in biomechanical failure of the digital support mechanism (Fig. 1).
- Normally, the equine hoof grows continuously due to proliferation of epidermal basal epithelial cells of the coronary tubules and proximal and distal lamellae, with minimal proliferation in the mid-lamellar region of the hoof.
- Horses with chronic laminitis often display impaired or abnormal hoof growth with excessive cellular proliferation of mid-lamellar epidermal epithelial cells (2).
- p63, a transcription factor necessary for epithelial stem cell proliferation in mice (3), might also regulate normal and pathological equine hoof growth.

## OBJECTIVE

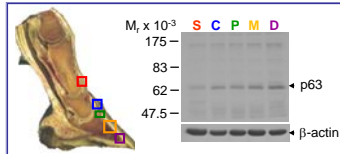
To verify and characterize the presence of equine p63 in epidermal epithelium.

## METHODS

- Tissue retrieval (Fig. 2):** Immediately following euthanasia, the distal phalanx was disarticulated at the metacarpophalangeal joint, and 1 cm wide slices were cut with a band saw. A scalpel was used to cut along the inner hoof wall and underlying bone. Dermal and epidermal tissue from the regions indicated in Fig. 2 were dissected and snap frozen in liquid nitrogen or fixed in Bouin's solution for paraffin-embedding or 4% paraformaldehyde for embedding and freezing in OCT.
- Protein immunoblotting (Fig. 3)** was analyzed by SDS-PAGE separation of proteins on 8% gels and electrophoretic transfer of proteins to (PVDF) membrane, cut at the 47.5 molecular weight marker, blocked with 5% fish gelatin followed by immunoblotting by standard methods of the top half with a mouse monoclonal anti-human p63 antibody (1:200, clone 4A4, Sigma, St. Louis) and the bottom half with a monoclonal anti-β-actin HRP-conjugate (1:5 x 10<sup>4</sup>, clone AC-15, Sigma).



**Fig. 1. Microanatomy of the equine foot.** Diagram showing the dermal papillae of the coronet and interdigitating epidermal and dermal lamellae of the inner hoof wall. Connective tissue attaches the distal phalanx (P3) to the basement membrane of the lamellar dermal/epidermal junction. The lamellar dermal/epidermal junction connects the skeletal elements to the hoof wall, suspending the horse's weight. The basement membrane is shown artificially detached to reveal the basal cells of the secondary epidermal lamellae beneath (1).

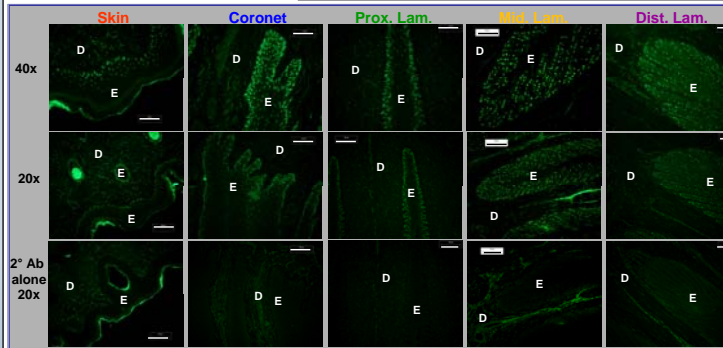


**Fig. 2. Tissue retrieval from equine foot.** Tissues sampled at the dermo-epidermal junction of the foot integument: S: Skin, C: Coronet, P: proximal lamellae, M: mid lamellae, D: distal lamellae.



**Fig. 3. Anti-p63 immunoblot of proteins extracted from different regions of the equine foot.** Proteins from snap-frozen tissue (S: skin, C: coronet, P: proximal lamellae, M: mid lamellae, D: distal lamellae) extracted with SDS sample buffer and subjected to 8% SDS-PAGE followed by immunoblotting with anti-p63 (clone 4A4, 1:200) and anti-β-actin (clone AC-15, 1:5 x 10<sup>4</sup>).

**Fig. 4. Lamellar histology:** Hematoxylin-Eosin stain of paraffin section (left) and indirect immunofluorescence of cryosection with anti-cytokeratin-14 antibody (right). Cytokeratin-14 is localized to the basal cells of the secondary epidermal lamellae. PEL: Primary Epidermal Lamellae; PDL: Primary Dermal Lamellae; SEL: Secondary Epidermal Lamellae; SDL: Secondary Dermal Lamellae.



**Fig. 5. Indirect immunofluorescence with anti-p63 antibody localizes p63 to the nuclei of basal and parabasal cells of the epidermal epithelium in several regions of the equine skin and hoof.** D: Dermal; E: Epidermal. Tissues as for Fig. 2, 3. Top row: 40x objective (400x), scale bar = 50 μm; Middle row: 20x objective (200x), scale bar = 100 μm; Bottom row: Secondary antibody alone (20x objective/200x).

- Indirect immunofluorescence (Fig. 4; 5)** was performed on Bouin's fixed, paraffin-embedded tissues (6 μm sections, Fig. 5) or PFA-fixed, OCT-embedded frozen tissues (10 μm sections, Fig. 4) using standard methods. Anti-p63 (clone 4A4) was used at 1:500 dilution, rabbit anti-human cyokeratin-14 (Abcam ab53115) was used at 1:50 dilution. Fluorescein (fitc)-conjugated secondary goat anti-mouse or goat anti-rabbit antibodies (Jackson ImmunoResearch, West Grove, PA) were used at 1:500 dilution. Slides were viewed under epifluorescence at 200x and 400x.

## SUMMARY OF RESULTS

- Anti-human p63 antibody recognizes a single protein band of the appropriate relative molecular mass in all tissues of the equine foot and adjacent skin. (Fig. 3).
- Anti-cytokeratin-14 antibody localizes to the cytoplasm of basal epithelial cells of the epidermal lamellae (Fig. 4).
- Anti-p63 immunoreactivity is localized to the nuclei of basal and parabasal cells of epidermal epithelium of skin, coronary tubules, and secondary epidermal lamellae in proximal, middle, and distal hoof regions (Fig. 5).

## CONCLUSIONS

- An equine p63 homolog is present in the nuclei of equine epidermal basal/parabasal epithelial cells of the skin and hoof.
- Normally quiescent regions of the hoof may have the capacity for proliferation during pathological processes such as laminitis.

## REFERENCES

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