

## Wound Healing Process of Uncomplicated “Rusterholz” Ulcer, Following Treatment by Wooden Block and Hoofgel® in Bovine Hoof: Histopathological Aspects

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**Abstract:** A broad and very detailed histopathological knowledge on cutaneous wound healing particularly in human skin is available, but few studies have documented this event in bovine claw sole ulcers. It seems the mechanism of wound healing might be basically the same followed by second intention healing which is finished in a period of time by formation of granulation tissue in damaged corium and subsequently completed by keratinized epidermis. This study was aimed for more histopathological information when wooden block and solka Hoofgel are applied as a treatment device for uncomplicated sole ulcers. Sixteen milking cows (age 2-5 years) which suffering from uncomplicated sole ulcer, following treatment by claw block (wooden block) on the healthy claw to reduce weight bearing on the affected claw and topical solka gel on affected claw from a farm in the vicinity of Tehran were considered. In all cases Lameness score was 3 (1-3 are ordinal scale), Lesion score was assessed 4 (1-4 are ordinal scale) and the mean lesion size was 16×9 mm. Regardless of the location's of the sole ulcers, The sampling sites were settled at caudo-medial part in the center sole namely “Specific ulcer site”. 16 safe ulcers in lateral claw of left rear foot, contained the corium and the part of the epidermis were collected in three time intervals 1,10,21 days and examined histopathologically. These samples were fixed in 10% buffered formalin and embedded in paraffin. Sections (3-5 µm thick) were taken from the paraffin-embedded tissue and stained with Hematoxylin and Eosin. Inflammatory changes were evident on day 1 because of disruption of the superficial dermis vessels. Some areas and horn layer surrounding the ulcer shows dilated tubules which were parallel to the sole and microcracks extending to the stratum spinosum. In day 10, the progressive epidermal regeneration from the edges of the lesion and reattachment between the over growing epidermis and the base membrane were seen. The proliferation in connective tissue was accompanied by the formation of new dermal vessels. The vascularization in the dermis was completed on day 21. The lesion was covered by moderate differentiated cornified epidermis. The dermal papillae showed marked mononuclear cells infiltrate. From these histopathological changes which is occurred during these stages of healing, it seems that by applying wooden block to the healthy claw to reduce weight bearing on the affected claw and using Solka hoofgel® which is a product, has some ingredient such as zinc and some organic acid, the time of wound healing process was decreased. So use of a modified treatment, influence on the healing process in macroscopical and histopathological aspect.

**Key words:** Wound healing process, histopathological aspects, horn layer, ulcer, bovine hoof

### INTRODUCTION

“Rusterholz” ulcer located in the region of the sole-bulb junction of the lateral claw of the hind leg, usually nearer the axial than the abaxial margin<sup>[1]</sup>. This ulcer is a perforation of the horn layer –develops when tissue necrosis is severe and extensive enough to hinder horn production permanently. Necrosis in the corium of the sole and therefore ulcers are usually focal. The resulting

lesions range from impaired horn production, with diffuse softening and discolouration in the horn of the sole<sup>[2]</sup>. The pathogenesis of “Rusterholz” ulcer is the circulatory disturbance in the corium due to primary damage and degeneration of the cells in the epidermis<sup>[3]</sup>. Localized contusion of the corium as a result of overloading and heel horn erosion is also considered to be a contributory factor in the development of sole ulcers in cattle.

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Mechanical pressure on the corium causes an ischemic necrosis of the corium. This necrosis lead to discoloration in the horn at the specific site because of poor-quality horn production and hemorrhage<sup>[4]</sup>. In human wound healing process, morphological healing are those that concern the Epidermis and those taking place with in the Dermis. Epidermal events are cell migration and epithelial cell proliferation. Dermal events are cellular infiltration, formation of granulation tissue and collagen production<sup>[5]</sup>. In the Wound healing process of the sole ulcers with out use of wooden block and hoof gel, the process of healing is similar to human wound healing<sup>[6]</sup> and the mean time for the formation of closed horn layer for uncomplicated sole ulcers which included application of claw block was 25 days when there were slight corium alteration and 48 days when severe changes were present<sup>[7]</sup>.

In this study the histopathological changes in the mechanism and time of wound healing process of sole ulcer after applying of wooden block on the healthy claw and using topical solka hoofgel (which contained some ingredients such as zinc and organic acids) on affected claw were examined.

## MATERIALS AND METHODS

In this study, 16 Holstein dairy cows (age 2 to 5 years) from a farm in the vicinity of Tehran which suffering from uncomplicated sole ulcer, following treatment by claw block (wooden block) on the healthy claw to reduce weight bearing on the affected claw and topical solka gel on affected claw were considered. In all cases Lameness score was 3 (1-3 are ordinal scale), Lesion score was assessed 4 (1-4 are ordinal scale) and the mean lesion size was 16× 9 mm. Regardless of the location's of the sole ulcers, The sampling sites were settled at caudo-medial part in the center sole namely "Specific ulcer site". 16 safe ulcers in lateral claw of left rear foot, contained the corium and the part of the epidermis were collected in three time intervals 1, 10, 21 days and examined histopathologically. These samples were fixed in 10% buffered formalin and embedded in paraffin. Sections (3-5 µm thick) were taken from the paraffin-embedded tissue and stained with Hematoxylin and Eosin.

## RESULTS AND DISCUSSION

Histopathological study on biopsy sections in days 1, 10 and 21 showed the following results.

**In day 1:** there were acute inflammatory changes due to disruption of the superficial dermis vessels. Red and White blood cells were scattered over the dermal and epidermal tissue. In the corium, congestion and edema

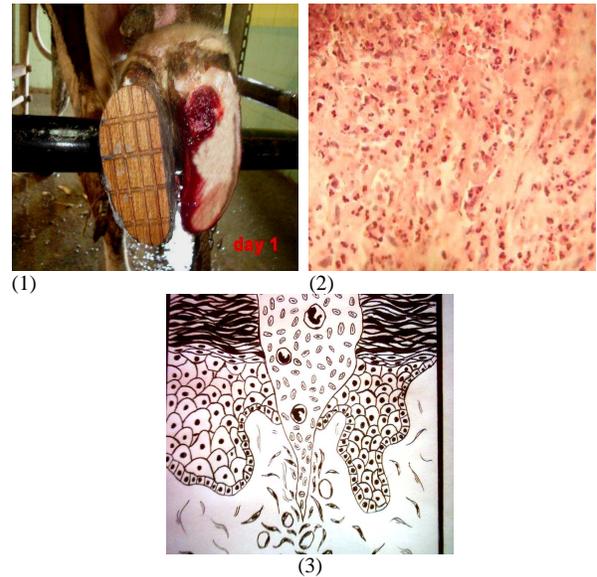


Fig. 1: In Day 1: 1: Sole ulcer after treatment, 2: Acute inflammatory changes, disruption of the superficial dermal vessels, 3: Schematic inset in dermis and epidermis showing the damaged tissues and the blood clot filling the defect

in connective tissue were seen. Dermal vessels were plugged by thrombi and the living epidermis next to the wound margin shows necrosis, loss the cells in the stratum basale and stratum spinosum and dyskeratotic changes. Some areas and horn layer surrounding the ulcer shows dilated tubules which were parallel to the sole and microcracks extending to the stratum spinosum (Fig. 1).

**In day 10:** The progressive epidermal regeneration from the edges of the lesion and reattachment between the over growing epidermis and the base membrane were seen. The wound surface was covered by a thin layer of softly cornified cells. But the segment specific differentiation of the epidermal cell was still disturbed. The proliferation in connective tissue was accompanied by the formation of new dermal vessels (Formation of granulation tissue) (Fig. 2)

**In day 21:** Vascularisation in the dermis was almost completed. Rete ridge formation in epidermis with acanthosis and parakeratotic hyperkeratosis (Degenerated cells) were seen. There were still areas with dyskeratosis resulting in a disturbed synthesis of keratin proteins and in attempt to close the ulcer, there was supra basal epidermal mitosis and proliferation of this layer, follows failure of normal proliferation and differentiation of the basal layer. Also massive proliferation of fibroblasts and vessels with

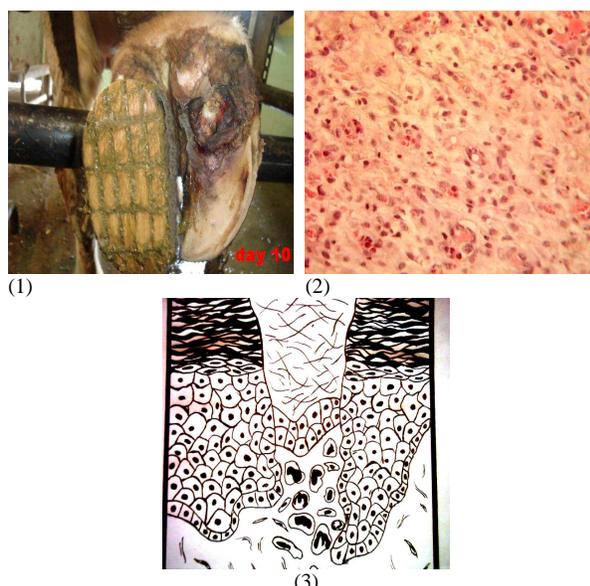


Fig. 2: In day 10: 1: Wound covered by a thin and soft layer of horn, 2: Formation of new dermal blood vessels (Granulation tissue), 3: Schematic inset showing the new generated epidermal cells covering the lesion

proliferation of tunica media in arterioles were seen. The lesion was covered by moderate differentiated cornified epidermis. The dermal papillae showed marked mononuclear cells infiltrate (Fig. 3).

In this study, histopathological evaluation shows that the mechanisms of wound healing in sole ulcer and cutaneous wound healing is basically the same. So we can see three parts of a wound: Inflammatory exudate, Granulation tissue formation and proliferation of connective tissue<sup>[8]</sup>. In this ulcer, the healing process was by secondary union ("second intention healing" by removing all dead tissue and necrotic debris and to fill in the tissue defect with vital cells), so the base and margins of the defect were first layered with granulation tissue and the size of the defect covered with epithelium<sup>[5]</sup>. The mean time for formation of closed horn layer for uncomplicated sole ulcers which included application of claw block was 25 days when there were slight corium alteration and 48 days when severe changes were present. And histopathological examination in cases of sole ulcers 20 days after initiation of treatment found cornification and maturation of epidermal cells, as well as formation of a new vascular bed in the dermis. Even after 50 days, histological integrity of the epidermis was still incomplete<sup>[7]</sup>. In this study, by applying wooden block to the healthy claw to reduce weight bearing on the affected claw<sup>[7]</sup> and using Solka hoofgel® which is a

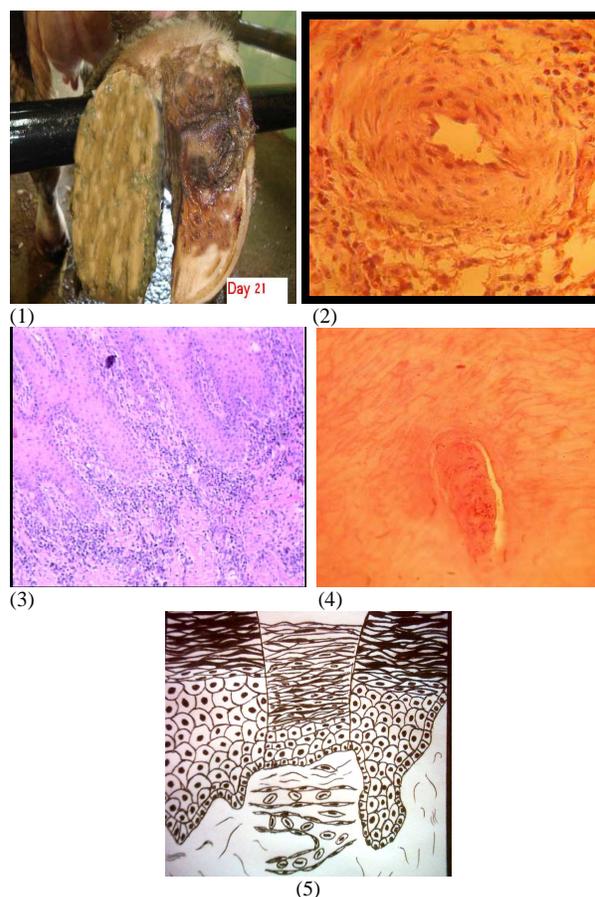


Fig. 3: In day 21: 1: lesion covered by horn completely, 2: proliferation of tunica media in an arteriolar, 3: Rete ridge formation in epidermis with acanthosis and parakeratotic hyperkeratosis, 4: The lesion was covered by mature horn layer, 5: Former lesion is filled by organized cornified epidermis, vascularization in the dermis is completed

product, has some ingredient such as zinc and some organic acids, the time of wound healing process was decreased. Zinc is necessary for the formation of many enzymes including nucleic acid polymerase and increase the rate of epithelization in the wound. Organic acids appears to effect the production of extracellular matrix of granulation tissue<sup>[5]</sup>. So by applying wooden block and use of topical hoofgel on affected claw, healing process of sole ulcer took 21 days to form a closed horn layer and after this time, histological integrity of the epidermis was completed and recurrence of sole ulcers was limited to all cases.

So use of a modified treatment, influence on the healing process in macroscopical and histopathological aspect.

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