



Arteriosclerosis in buffalo hooves—an age related change or a pathology of lameness?

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ABSTRACT

Histopathological studies of the hoof horn tissue from the solar surface were undertaken in mature buffaloes of third or more lactation after taking biopsy. Biopsy samples were taken either of the left or right hind lateral digit due to bilateral symmetry of lesions in hind lateral claws. Five of the animals were having sole hemorrhages with 1 buffalo having overgrown hoof while the other one was having under run sole in addition to sole hemorrhages in the hind lateral digits. Other 5 animals were having perfectly normal hooves with no lesions in their hind lateral claws and were kept as healthy control. There was mild to marked proliferation of tunica media of arterioles (arteriosclerosis) in the corium resulting in partial to complete occlusion of the lumen of blood vessel in animals having sole hemorrhages. Similar alterations were also observed in the animals having healthy hooves with no lesion. Besides that, mild focal lymphomononuclear cell infiltration was also seen in the periarteriolar area along with perivascular edema and fibroplasia in both groups. In buffaloes with overgrown hooves, the only additional change was the presence of occasional keratin bodies in stratum spinosum layer of the hoof epidermis. It may be inferred that there was no significant histopathological differences between healthy hooves and hooves having sole hemorrhages except degenerative changes in the stratum corneum and keratin bodies in stratum spinosum layer of the epidermis in the hooves with sole lesions. Arteriosclerosis, which was considered as a typical feature in the pathogenesis of sole lesions of lameness in cattle might be an age related change not putatively a pathology of lameness in buffaloes. Moreover this is the first histopathological study on lameness in buffaloes.

Key words: Arteriosclerosis, Buffalo, Laminitis, Pathogenesis

Lameness in dairy animals is a major cause of involuntary culling after mastitis. About 90% of lameness in dairy cattle (Clarkson *et al.* 1996) and buffaloes (Randhawa 2006) occurs due to sole lesions. Kalsi *et al.* (2002) reported 59.32% incidence of overgrown hooves in buffaloes. Clinical lameness in buffaloes is nearly 2% but the lesions of subclinical laminitis are up to 46% (Randhawa 2006). Sole hemorrhages were observed as the predominant lesion (16.32%) in buffaloes at organized farms whereas underrun soles was the major contributing lesion at unorganized farms (Randhawa 2006).

Arteriosclerosis, observed as common histopathological change in bovine pododermatitis aseptica diffusa, has been supporting the theory of hypoxia and ischemia (Mgasa 1987,

Boosman *et al.* 1990, Singh *et al.* 1992). Hyperemia, oedema, hemorrhages and thrombi were other important features in acute laminitis (Nilsson 1963, Maclean 1971, Andersson and Bergman 1980). In chronic disease, vascular changes of arteriosclerosis with proliferation of tunica media (Boosman *et al.* 1989) dominate. Histopathological alterations causing foot lesions were evaluated in cattle but studies are not available on histopathological alterations in epidermis and dermis of hooves in buffaloes bearing sole lesions. This is the first effort to investigate whether histopathological alterations in hooves of Murrah buffaloes having sole lesions are similar in cattle or vary from the buffaloes having normal hooves with no sole lesions. So the study was especially planned on the present herd with 16.32% prevalence of sole hemorrhages.

MATERIALS AND METHODS

Lactating Murrah buffaloes (5) with sole hemorrhages of severity (2) on the severity scale in hind lateral claws were randomly selected from the lactating dairy herd of the University, while undertaking epidemiological studies on lesions of foot lameness. One of the animals from these 5

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animals was having overgrown hooves and other one was having under run soles in addition. Other 5 buffaloes with normal healthy hooves from the same herd were taken as control. Biopsy samples were collected from either right or left lateral claw of all the animals because mostly the lesions were symmetrical.

Severity scale: Following scale was used (Randhawa 2008).

Severity 1: General reddening of sole or small petechial or ecchymotic haemorrhages.

Severity 2: Visible hemorrhages distributed in half of the sole or bright red spots in a particular area.

Severity 3: Visible hemorrhages distributed in more than half of the sole or dark red spot in a particular area.

Biopsy technique

Biopsy technique of Singh *et al.* (1993) was used. After restraining the animal in a hoof-paring crate, the hoof was cleaned, pared and thoroughly disinfected with povidone-iodine solution. Anaesthesia of the foot was achieved by injecting 15 ml of 2% lignocaine hydrochloride about 2" above the interdigital cleft at the planter aspect. A hole was carefully made in the sole at the ulcer site (sole bulb area) using a hand drill with 7 mm bit to remove most of the horn tissue leaving a thin layer of horn covering the corium. This could be judged by observing the pinkish tinged corium that became visible through the horn. A 3 mm biopsy punch was used to penetrate through the horn into the corium. After inserting it up to its full length, 8–10 rotations were given and the punch was withdrawn (Fig.1). The biopsy tissue was removed and put into 10% formalin for fixation.

After taking biopsy, the hole was plugged with sterile gauge and finally plugged with plaster of paris. A short 3–day course of streptomycin and penic along with anti-inflammatory drugs was given to reduce the risk of infection. All the animals except to those required treatment for 1 or 2 week, were cured in 3 days.

Processing and staining of tissues for histopathology

The relevant tissue samples were fixed in 10% formalin and were dehydrated in ascending grades of alcohol. The tissues were embedded in liquefied wax, and blocks were

prepared. Five-micron thick sections were cut for routine staining. The sections were dewaxed using xylene and hydrated through alcohol and water (Luna 1968) and staining was done as per standard haemotoxylin and eosin staining method.

RESULTS AND DISCUSSION

Laminitis is believed to be a multifactorial disease. The controversy regarding exact etiology and precise pathogenesis of laminitis still prevails. Two schools of thought originate from the previous histopathological studies that whether the cause of laminitic lesions is hypoxia and ischemia (Nilsson 1963) or stagnant hypoxia (Boosman 1990).

In the present study, arteriosclerosis with varying degree of proliferation of tunica media of the arterioles has been observed in corium of four of the five animals in digits having sole hemorrhages (Table 1; Fig.2). These findings were similar to the earlier findings of histopathological alterations in dairy cattle (Boosman *et al.* 1989, Singh *et al.* 1992, Mochizuki *et al.* 1996). Almost similar changes were observed in buffaloes with normal healthy digits with no lesions (Table 2; Fig 3). Besides that 2 of the 5 animals having sole hemorrhages and 3 out of 5 animals having healthy hooves were having mild infiltration of lymphomononuclear cells (Fig. 2) and one each from both groups was having neutrophilic infiltration. Two of the biopsy sections from healthy hooves and 3 of the sections from hooves with sole hemorrhages were having varying degree of fibroplasia. Three of the 5 biopsy samples from animals with sole hemorrhages in their hooves were having partial to complete loss of stratum corneum along with degenerative changes in the epidermis. Edema was evident in 3 sections each from the affected hooves (Figs 2,7) and healthy hooves (Fig. 4). In an animal having overgrown hooves along with sole hemorrhages, keratin bodies were observed in the stratum spinosum layer of the epidermis (Figs 5,6)

Two types of hypothesis can be drawn from these results. One indicating that arteriosclerosis is an age related change with no relationship with subclinical laminitis because it is evident both in buffaloes having healthy digits and buffaloes with sole hemorrhages in their digits. If arteriosclerosis is



Fig. 1. Biopsy taking procedure in a buffalo hoof.

Table 1. Histopathological findings in buffalo hooves with sole hemorrhages and additional lesions

Status of lesions	Arterio-sclerosis	Keratin bodies in stratum spinosum	Infiltration of neutrophils	Edema	Vascularisation	Fibro-plasia	Degenartive changes in St. corneum	Infiltration of mononuclear cells
SH+OH	+	+	+	+	mild	mild	-	-
SH	-	-	-	-	-	-	+	-
SH+US	+	-	-	+	-	marked	-	-
SH	+	-	-	+	-	-	+	+
SH	+	-	+	-	mild	mild	+	+

SH, Sole hemorrhages; SH+OH, sole hemorrhages and overgrown hooves; SH+US, sole hemorrhages and under run soles.

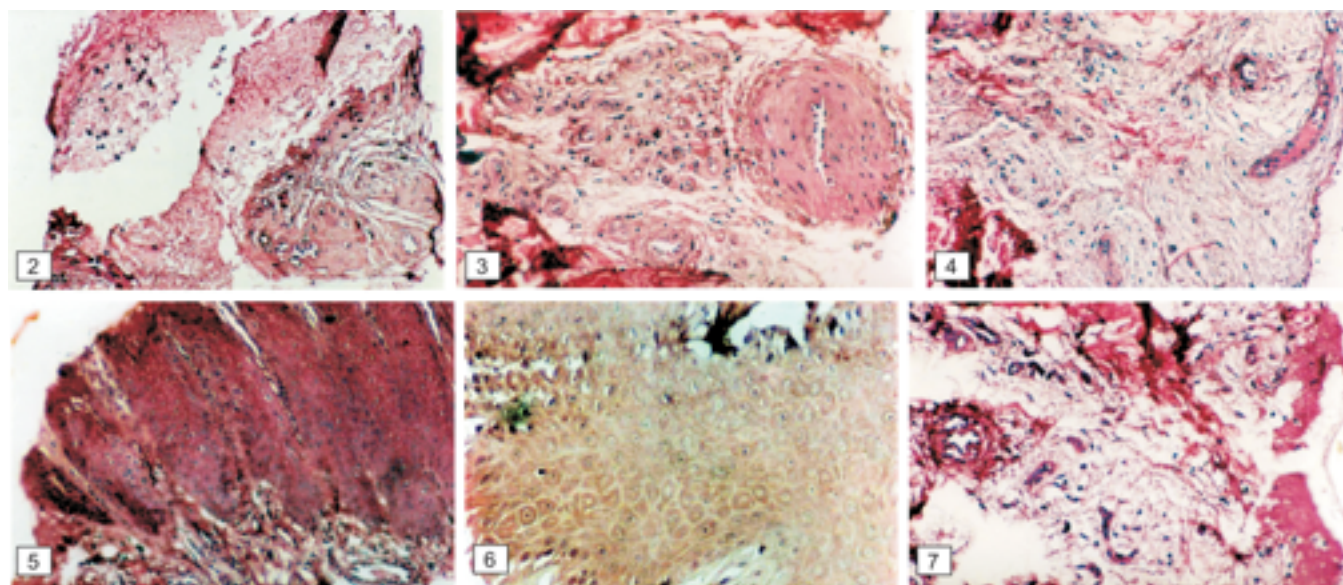
Table 2. Histopathological findings in normal healthy buffalo hooves with no lesions

Arterio-sclerosis	Keratin bodies in stratum	Infiltration of neutrophils spinosum	Edema	Vascularisation	Fibro-plasia	Degenartive changes in St. cornium	Infiltration of mononuclear
+	-	-	-	-	mild	-	+
+	-	-	+	-	-	-	+
+	-	-	-	mild	-	-	-
-	-	-	-	-	marked	+	+
+	-	+	+	-	-	-	-

not a pathology of lameness in the present study then it might be suggesting that it is not the ischemic hypoxia due to arteriosclerosis which, is responsible for lesions of subclinical laminitis rather it might be vasodilation and AV shunts which might have caused stagnant hypoxia (Boosman 1990)

resulting in the sole lesions in buffaloes in this study.

The other hypothesis could be that laminitic changes in the dermis might have occurred few days before the sampling time so unable to be observed as lesions of sole hemorrhages in epidermis of claws in buffaloes with healthy digits because



Figs 2–7. **2.** Arteriosclerosis of blood vessels, edema and degenerative changes with lympho-mononuclear cell infiltration in buffalo hooves with lesions of sole hemorrhages and overgrown hooves (H.E.×150). **3.** Marked thickening of tunica media (A) along with congestion (B) of blood vessels in the dermis of healthy buffalo hooves having no sole lesion (H.E.×150). **4.** Chronic changes characterized by fibroplasia (F) and edema in the corium in healthy buffalo hooves with no sole lesion (H.E.×150). **5.** Keratin bodies (K) in stratum spinosum layer of the epidermis along with lamellar and papillar junction in buffalo hooves with lesions of sole hemorrhages and overgrown hooves (H.E.×75). **6.** Keratin bodies (K) in stratum spinosum layer of the epidermis in buffalo hooves with lesions of sole hemorrhages and overgrown hooves (H.E.×150). **7.** Mild lympho-mononuclear infiltration, edematous changes along with degeneration of epithelium in buffalo hooves with lesions of sole hemorrhages (H.E.×150).

horn growth is approximately 5 mm per month (Vermunt and Greenough 1995) and it may take approximately 3 months for lesions to reach the solar surface. These facts seem further justified by other changes associated with subclinical laminitis in present study, such as infiltration of lympho-mononuclear cells (Maclean 1971), mild infiltration of neutrophils, mild to marked fibroplasia in the corium of the both healthy and digits with sole hemorrhages. Similar substantial perivascular lymphocytic infiltration (Maclean 1971) and focal infiltration of neutrophils have also been observed along with acidophilic or keratin bodies in epidermal laminae (Nilsson 1963, Maclean 1971, Andersson and Bergman 1980, Mochizuki *et al.* 1996) in the previous studies. Edematous changes in both types of digits further support the previous findings. Degenerative changes in the stratum corneum of the epidermis were more marked in the digits having sole hemorrhages as compared to healthy digits. Mgasa (1987) observed degenerative changes in addition to arteriosclerosis as pathology of bovine pododermatitis aseptic diffusa. These degenerative changes may be suggesting that sole hemorrhages /subclinical laminitis if not checked at proper time may lead to formation of sole ulcers due to degeneration of epidermal layers and exposure of underlying corium. That might be a reason that degenerative changes in the stratum corneum were observed in one healthy digit out of five with no lesion. Mast cells were not observed in any of the biopsy sample as observed in previous studies (Nilsson 1963, Maclean 1971, Andersson and Bergman 1980).

In one of the biopsy sample from affected digits having overgrown hoof in addition to sole hemorrhages, the characteristic finding of laminitis i.e. keratin bodies were observed in the stratum spinosum layer of epidermis (Nilsson 1963, Mclean 1971, Andersson and Bergman 1980). Andersson and Bergman (1980) hypothesized that the circulatory change in the hooves caused an insufficient nutrient supply to the keratin producing cells with a synthesis of structurally incompetent keratin. The occasional keratin bodies observed in the stratum spinosum of epidermis of buffalo hooves in the present study fully endorses this hypothesis of the researchers. The other digit having underrun sole in addition to sole hemorrhages were having similar findings as the digits having only sole hemorrhages and normal healthy digits. This might be indicative that sole hemorrhages and underrun soles have similar pathophysiology of their occurrence. The present study seems to agree with previous studies that vascular changes are primarily followed by secondary epidermal changes (Nilsson 1963) leading to laminitis which results in production of poor quality horn which is an important predisposing factor to claw disorders such as sole hemorrhages, sole ulcers, overgrown and underrun soles and white line lesions (Maclean 1971, Colam-Ainsworth *et al.* 1989, Greenough and Vermunt 1990, Randhawa 2006).

It may be concluded from the present study that contrary

to the previous studies arteriosclerosis may not be a pathological lesion of lameness in buffaloes rather the hypoxia caused in the digits could be stagnant hypoxia which might have occurred due to changes like vasodilatation and AV shunting of blood bypassing the digits. The hypothesis that arteriosclerosis is an age related change in buffaloes has to be confirmed further by planning a long term study on the same group of animals from birth up to the time they reach third or further lactation age.

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