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Equine laminitis: Bites by *Bothrops spp* cause hoof lamellar pathology in the contralateral as well as in the bitten limb

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Abstract

The envenoming caused by *Bothrops* snakebite includes local symptoms, such as pronounced edema, hemorrhage, intense pain, vesicles, blisters and myonecrosis. The principal systemic symptom consists in the alteration of blood clotting, due to fibrinogen consumption and platelet abnormalities. The horses involved in this study had this symptomatology and one of them exhibited symptoms consistent with laminitis in the bitten and in the contralateral limbs. Laminitis lesions were characterized by separation of the hoof lamellar basement membrane (BM) from basal cells of the epidermis. These results demonstrated that *Bothrops* snake venom can induce acute laminitis. We conclude that components of the venom, probably metalloproteinases, cause severe lesions in the hoof early in the envenoming process. Antivenom therapy must be initiated as soon as possible in order to prevent complications, not only to save the life of an envenomed horse, but also to avoid the dysfunctional sequels of laminitis.

Keywords: Horse; Laminitis; Basement membrane; Snake venoms

1. Introduction

Northeastern Argentina is characterized by the management of beef cattle in extensive areas of natural grassland. Horses are an integral part of the management system but unfortunately many poisonous snakes inhabit the same area. They are a common cause of envenoming in cattle and horses. All the envenomed horses described in this study were from country areas.

Many Elapidae and Viperidae inhabit northeastern Argentina, and the dangerously venomous ones are a serious threat to the well-being of both men and animals (Esteso, 1985). *Bothrops* snakes (yarará) are the most frequent cause of envenoming and of these *Bothrops alternatus* (víbora de la cruz)

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is widespread in the region of the country (Esteso, 1985), where horses play an important role in farm activities.

Snake venom is a complex mixture of many compounds that vary in composition and quantity, depending on the species, age of the animal, geographical area and season. South American *Bothrops* venoms have components that induce systemic hemorrhage, coagulation disorders, cardiovascular shock and acute renal failure (Bolaños, 1982; Chaves et al., 1989; Gutiérrez and Lomonte, 1995). Complex local effects, such as hemorrhage, edema and myonecrosis may also be present in the envenomed animal (Acosta de Pérez et al., 1998; Teibler et al., 2001; Gutiérrez and Lomonte, 1989).

Lameness (claudication) and dysfunction of the bitten limb has been observed in horses after natural snakebite presumably because of the rapid effect of the venom in the bitten area. Chronic laminitis has been listed as a sequel to snakebite in horses (Acosta de Pérez and Brem, 1997).

The objectives of this study were to demonstrate that venom from *Bothrops* snakes may cause acute laminitis in horses. We determined if the dermo–epidermal interface of the equine hoof wall was affected during the initial stages of the envenoming, after venom enters bloodstream and it is distributed throughout the organism. We hoped that this information would encourage early diagnosis that would not only save the life of the patient but also avoid the crippling consequences of distal limb dysfunction.

2. Material and methods

2.1. Animals

Four horses from country areas, all naturally envenomed, were the subjects of this study. Two animals were taken at the Veterinary Hospital of The School of Veterinary Medicine, Universidad Nacional del Nordeste, but did not receive antivenom treatment because the antivenom was not available in the healthcare centre and during the search of this, the animals died.

The other two horses were observed in the field, where the accident happened, and they did not receive antivenom treatment either because we did not have this last. All of them died due to the poisoning and in the two Veterinary Hospital cases, immediately after death, both the bitten and the contralateral limbs were amputated. In the two field cases only the bitten limbs were processed. Clinic symptoms and hematology tests were performed to confirm the diagnosis of snake envenomation.

The horses had been bitten on one of their limbs, two of them over the dorsal metacarpus/tarsus, and two above the coronet of the hoof. The time between envenomation and death ranged from 24 to 36 h.

Normal tissue samples (controls) were provided by the Pathology Laboratory of The School of Veterinary Medicine, Universidad Nacional del Nordeste (UNNE).

2.2. Blood samples

Blood was obtained by jugular venepuncture and used to determine clotting time for each bitten animal. Clotting time was determined as the time needed for formation of a firm clot from recently obtained blood collected in standardized test tubes at 37 °C (Lee and White, 1913; Tocantins and Kazal, 1964).

2.3. Treatment

The Hospital treated animals were given 1.1 mg/kg flumixin meglumine intravenously for the treatment of inflammation combined with saline fluid therapy to reverse intense dehydration awaiting the arrival of the antivenom. However, both horses died 3–4 h after being admitted to hospital. The field cases received no therapy as they were close to death. All the animals showed clinic symptoms of systemic haemorrhagic.

2.4. Histopathological study of hoof samples

Limbs were disarticulated at the metacarpal-phalangeal joint and sectioned with a bandsaw, following the protocol of Pollitt (1996). Tissue blocks of the inner hoof wall were fixed in 10% formalin, dehydrated in alcohol and embedded in paraffin wax. Sections of 5 μm-thick were cut and stained with hematoxylin and eosin (H&E) and periodic acid Schiff (PAS) reagent and examined with a Leitz light microscope.

3. Results

All the animals studied in this work showed clinical signs typical of bites from the *Bothrops* snakes of Argentina. Thus there was extensive limb

edema, bleeding at bite site and intense pain (Fig. 1). In all cases, blood did not clot, even after 24 h of storage at room temperature.

3.1. Light microscopy of bitten and contralateral limbs

Histological analysis of controls, showed histology consistent with description of Pollitt (1994). Thus, the hoof wall dermal–epidermal interface consisted of dermal lamellae characterized by primary dermal lamellae (PDL) and secondary dermal lamellae (SDL). Interdigitating with these and complementary to them were the epidermal lamellae with their characteristic primary epidermal lamellae (PEL) and secondary epidermal lamellae (SEL). The tips of the SELs, as outlined by the PAS stained basement membrane (BM), were always rounded and never tapered or pointed. The SEL had basal cells with oval nuclei oriented at right angles to the long axis to the keratinised axis of the SEL. Dermal lamellae contained numerous blood vessels important for the exchange of oxygen and carbon dioxide, as well as for the supply of nutrition. The most important components of the lamellar BM are type IV and VII collagen fibers and laminin, which stained readily with PAS stain. Thus the BM stained as a fine, magenta colored line. The BM of normal lamellae penetrated deeply into the crypt between pairs of SELs and clearly outlined the tapered tip of each SDL (Fig. 2).

Fig. 1. Horse bitten by *Bothrops* snake in the fetlock of the posterior limb. Edema can be observed in the buttock area. It was assisted in the country. Besides local symptoms, it shows bleeding in areas where the horse was bitten by snake.

Three horses showed histopathological changes in the lamellar hoof wall, possibly as consequence of the action of the poison. The pathological changes observed were edema, hydropic degeneration with displacement of SEL cell nuclei. In one horse the lesions of laminitis (Pollitt, 1996) were present in both in the bitten and contralateral limbs. H&E stained sections showed SELs with a wavy appearance with SEL tips more pointed than normal. Some of the epidermal cell nuclei were pyknotic and others were round instead of oval and abnormally orientated with their long axes parallel to the long axis of the SEL. PAS stained sections showed SELs with the BM separated from the basal cells of the epidermis (Fig. 3).

4. Discussion

Snake venom poisoning is a complex phenomenon, in which many components participate and interact. About the studied cases in the present work, clinical symptoms and hematology test have taken us to confirm that all horses were envenoming by *Bothrops* snake, according with previous reports of Bothropic envenoming in humans (Esteso, 1985), dogs (Koscinczuk et al., 2000) and horses (Teibler et al., 2002), we demonstrate that the intoxication of the four horses was caused by the *Bothrops* snakes of Argentina. This assumption was, based on the local signs, present in all the animals, as well as systemic ones, such as unclottable blood due to fibrinogen consumption. These results are in accordance with previous reports of Bothropic envenoming in humans (Esteso, 1985), dogs (Koscinczuk et al., 2000) and horses (Teibler et al., 2002).

Structural changes were observable at the hoof lamellar dermal–epidermal interface, consisting of narrowing and loss of the normal disposition of SELs. Nuclei lost their normal position, and a considerable separation of the BM from the epidermic basal cells was evident in one of the cases. These results are similar to those observed in

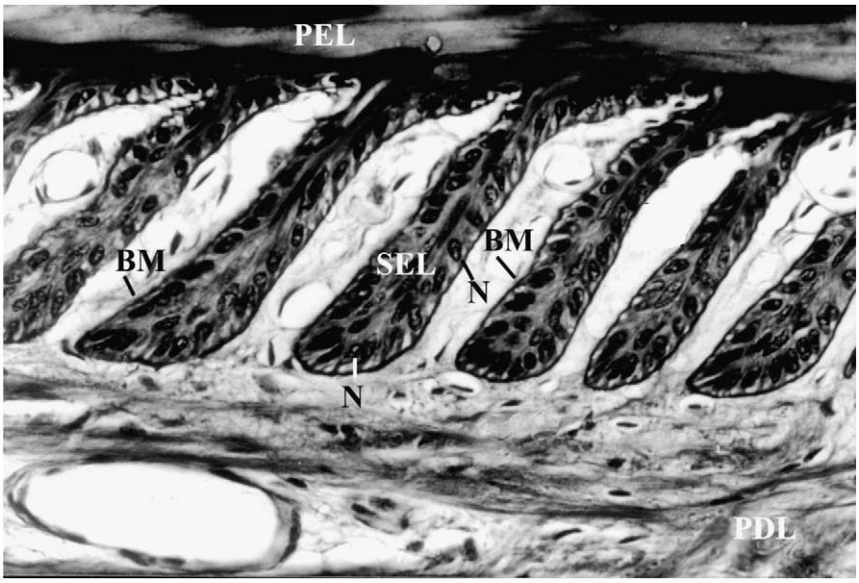

Fig. 2. Histology study of the dorsal hoof wall from equine normal, observed with light microscope. The basement membranes (BM) is closely adhered and stained as black line to the basal cells of the secondary epidermal lamellae (SEL). The SEL have basal cells with oval nucleus (N). Primary epidermal lamellae (PEL). Primary dermal lamellae (PDL). Sections were stained with PAS. Scale bar represents 10 μm.

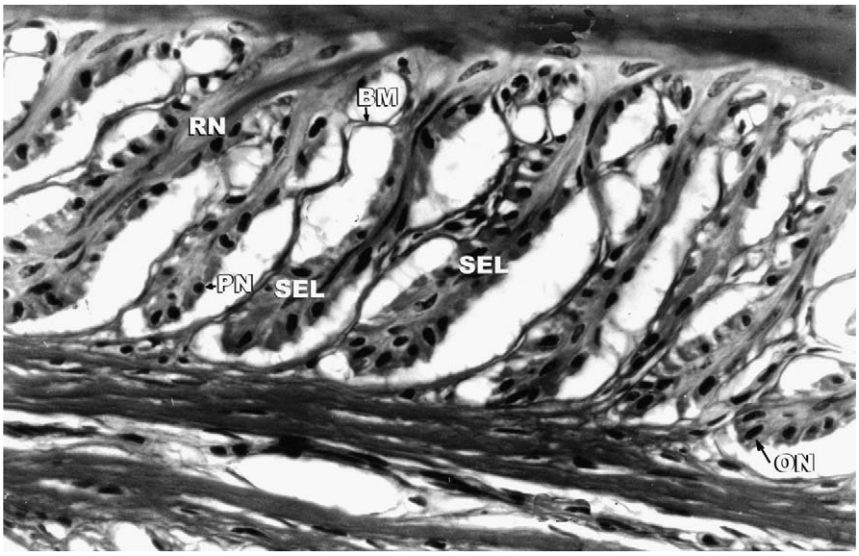

Fig. 3. Histopathological study of the dorsal hoof wall of contralateral limb to the bitten limb from equine attacked by *Bothrops* snake. Lesions characterized by separation of the SEL from their basement membranes (BM). Some of the epidermal cell nuclei were pyknotic (PN) and others were round (RN) instead of oval (ON) and were abnormally orientated with their long axes parallel to the long axis of the SEL. Sections were stained with PAS. Scale bar represents 10 μm.

vitro studies of venom activity on horse hoof (Pollitt et al., 1998).

In this work, laminitis was diagnosed by microscopy in both the affected and contralateral limbs, this suggesting a systemic action of the venom.

In particular, detachment of the BM alters the normal organization of the derma–epidermal junction. The latter is extremely important, as it keeps the hoof joined to the distal phalanx. The first consequence of this alteration is the displacement of

the distal phalanx inside the hoof, because of weight bearing and the traction generated by the deep digital flexor tendon on this bone. Finally, the suspensory attachment between hoof and distal phalanx fails (Pollitt, 1994). The present confirms development of acute laminitis secondary to *Bothrops* snakebite within 36 h of envenomation.

These results agree with a previous report about a horse bitten by *B. alternatus* from northeastern Argentina. The venom was inoculated with both fangs into the tissue over the dorsal left fetlock. The horse recovered after administration of antivenom, although local tissue alterations developed, including chronic laminitis, that caused the animal to claudicate and thus be less effective at work (Acosta de Pérez and Brem, 1997).

Laminitis after Bothropic envenoming is due to the action of the components of the venom, which act synergistically, strengthening the toxic effect. Hyaluronidase facilitates diffusion, and hemorrhagins affect vascular permeability (Gutiérrez and Rucavado, 2000). Hemorrhagins also affect fibrinogen, causing alteration of coagulation, platelet aggregation and interactions with platelet receptors (Kamiguti et al., 1996). Phospholipase A2 (PLA2) causes destabilization of membranes, altering permeability and hydrolysis of the bilayer (Gutiérrez and Lomonte, 1995).

Previous studies using scanning electron microscopy of hoof explants, exposed to the action of PLA2 from *B. alternatus* of Argentina, showed mild alteration of the secondary epidermic lamella and basal membrane (Teibler et al., 2002). As shown by this study the effects caused by the whole venom were more intense, suggesting that PLA2 is not the only factor responsible for the laminitis caused by snake venom. The development of equine laminitis is closely linked to the activation of metalloproteinases (MMPs) normally present in the basal cells of hoof wall lamellae (Kyaw-Tanner and Pollitt, 2004). It is possible that the laminitis in the foot contralateral to the one bitten, in one of the horses in this study, resulted from the hematogenous delivery of *B. alternatus* venom MMP (Cominetti et al., 2003). The MMP component of *B. alternatus* venom induces substrate detachment of keratincytes in vitro and this effect could have activated the process of basal cell dysadhesion and BM separation to cause laminitis in the contralateral foot of the horse described in this study.

We conclude that laminitis equine might be developing by *Bothrops* envenomation. It is important to highlight that the injury is present not only in the bite site but also in the other limbs.

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