ELSEVIER

Toxicon 48 (2006) 307–312

TOXICON

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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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Received 27 October 2005; accepted 1 June 2006

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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0041-0101/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.toxicon.2006.06.010

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