




Ophidian accident in horses in the Amazon Biome, Pará, Brazil¹

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ABSTRACT.- Serruya A., Lopes C.T.A., Silveira N.S.S., Barbosa C.C., Costa P.S.C., Duarte M.D., Campo K.F. & Barbosa J.D. 2024. **Ophidian accident in horses in the Amazon Biome, Pará, Brazil.** *Pesquisa Veterinária Brasileira* 44:e07411, 2024. Hospital Veterinário, Instituto de Medicina Veterinária, Universidade Federal do Pará, BR-316 Km 61, entrada pelo Instituto Federal do Pará, Castanhal, PA 68741-740, Brazil. E-mail: analielserruya.as@gmail.com

Bothrops atrox is the most common viper in the Amazon, and its venom causes local and systemic changes. This report describes the clinicopathological and laboratory findings of ophidism due to *Bothrops* in six horses in Pará, Brazil. The animals, which belonged to five different rural properties, showed clinical signs of apathy, anorexia, and increased touch sensitivity in areas of increased volume. Three animals were bitten in the distal part of the pelvic limbs and three in the head area. The affected animals in the distal limb area exhibited marked edema extending from the fetlock to the thigh and had difficulty moving. Those affected in the head region exhibited an increase in volume that gave the appearance of a “rhinoceros head,” as well as blackening of the mucosa of the lips and gums. All animals exhibited tachycardia and tachypnea, and laboratory findings of two animals showed anemia, leukocytosis, increased clotting time, and elevated liver (AST and GGT) and kidney enzymes (urea and creatinine). Treatment was ineffective, and three of four treated animals died. Necropsy was performed on three animals, revealing extensive hemorrhage in the tissues at the snake bite sites and incoagulable bloody fluid in the cavities. Congestion was observed in the diaphragm, in the serosa of the small intestine, and in lighter areas on the renal surface. Histopathology showed muscle degeneration, necrosis, acute tubular necrosis, hemorrhage, and hyaline casts in the kidneys. This case report highlights the clinicopathological findings of snakebite in horses. In addition, this seems to be the first report of bothropic envenom in a mule in Brazil.

INDEX TERMS: Ophidism, Amazon, mule, horses, clinicopathological aspects.

RESUMO.- [Acidente ofídico em equinos no Bioma Amazônico, Pará, Brasil.] *Bothrops atrox* é a serpente mais comum na Amazônia, e seu veneno provoca alterações locais e sistêmicas. Este relato descreve os achados clínico-patológicos e laboratoriais de ofidismo por *Bothrops* em seis

equídeos no Pará, Brasil. Os animais, que pertenciam a cinco propriedades rurais diferentes, apresentaram sinais clínicos de apatia, anorexia e aumento da sensibilidade ao toque em áreas de aumento de volume. Três animais foram picados na parte distal dos membros pélvicos e três na região da cabeça. Os animais afetados na área dos membros pélvicos apresentaram edema acentuado que se estendia do boleto até a coxa e exibiam dificuldade de locomoção. Aqueles afetados na região da cabeça exibiam aumento de volume que conferia a aparência de uma “cabeça de rinoceronte”, bem como escurecimento da mucosa dos lábios e gengivas. Todos os animais apresentaram taquicardia e taquipneia, e os achados laboratoriais de dois animais evidenciaram anemia, leucocitose, aumento do tempo de coagulação e aumento das enzimas hepáticas (AST e GGT) e renais (ureia e

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creatinina). O tratamento foi ineficaz, e três dos quatro animais tratados faleceram. A necropsia foi realizada em três animais e foi observada hemorragia extensa no tecido dos locais da picada da serpente e líquido sanguinolento incoagulável nas cavidades. Observou-se congestão no diafragma, na serosa do intestino delgado e áreas mais claras na superfície renal. A histopatologia mostrou degeneração muscular, necrose, necrose tubular aguda, hemorragia e cilindros hialinos nos rins. Este relato destaca os achados clínico-patológicos de picada de serpente em equídeos. Além disso, parece ser o primeiro relato de envenenamento por *Bothrops* em uma mula no Brasil.

TERMOS DE INDEXAÇÃO: Ofidismo, Amazônia, mula, equino, aspectos clínico-patológicos.

INTRODUCTION

Snakebites are a controversial issue in Brazil, but some authors consider them essential in Brazilian livestock due to their frequency and the economic losses they cause (Raposo et al. 2001, Cintra et al. 2014). However, statistics on snakebites in domestic animals could be more extensive and accurate, highlighting the need for further studies (Barni et al. 2012).

Many of the farm deaths in Brazil are attributed to snakebites. However, according to Pacheco & Carneiro (1932), rural people erroneously consider poisoning from plants and snakebites as the main causes of "sudden death" in animals. According to Tokarnia & Peixoto (2006), snakebite accidents in livestock are often overestimated because diagnosis is presumptive and based on assumptions and beliefs.

Snakes are classified in the kingdom Animalia, phylum Chordata, class Reptilia, order Squamata, and the suborder Ophidia and belong to several families. The family Viperidae includes the genera *Bothrops* (jararaca), *Crotalus* (rattlesnake), and *Lachesis* (surucucu); the family Elapidae by the genus *Micrurus* (coral snake); and the family Colubridae by the genera *Philodryas* (green snake) and *Clelia* (black or muçurana snake) (Fraga et al. 2013, Bernarde 2014, Monaco et al. 2017).

In Brazil, *Bothrops* spp. account for 88 to 89% of ophidian accidents. Ophidia of this genus inhabit humid places, plantations, pastures, and places uninhabited by humans. They are nocturnal, feed mainly on small rodents, and attack suddenly, lifting the anterior third of the body unnoticed (Grunert & Grunert 1969, Bernarde 2014).

Bothrops atrox (jararaca, Northern viper) is the most common viper in the Amazon and undoubtedly the main cause of accidents in the northern region where it lives (Bernarde 2014, Monaco et al. 2017, Moraes et al. 2021). Sporadic accidents with snakes of the *Bothrops* genus occur in cattle and horses (Tokarnia & Peixoto 2006, Silva et al. 2011); however, cases are rarely referred to laboratories to determine the cause of death (Grunert & Grunert 1969).

Domestic animals (livestock) susceptibility to *Bothrops* venom follows the following descending order: Horses, sheep, cattle, goats, pigs, and cats (Araujo & Belluomini 1960-62, Bolon et al. 2019). Animals are mainly stung on the head due to their curious behavior, but bites on the limbs are also common. Fatal accidents in large animals are less common because more venom is required than in small animals (Grunert & Grunert 1969, Tokarnia & Peixoto 2006, Radostits et al. 2020).

The venom of ophidians of the genus *Bothrops* contains a complex mixture of enzymes, peptides, and low molecular mass proteins with specific chemical and biological activities (Santos et al. 2016). Its composition generally contains hyaluronidase, which explains the speed of absorption by dispersion between tissues; hemotoxins and cytolytins, which cause local inflammation, necrosis, and damage to vascular epithelium; phospholipase A and esterase, which alter membrane permeability and release histamines and bradykinins. The venom also has proteolytic or necrotizing, coagulant, hemorrhagic, and nephrotoxic effects, leading to local and systemic changes (Méndez 1998, Gutiérrez et al. 2017).

There are many publications on human ophidian accidents in Brazil and the Amazon. Nevertheless, there are few reports of these accidents in livestock in this region. Ecological imbalance and exposure of animals in newly opened grazing areas are factors that favor these accidents. Considering the abundance of dangerous snakes in this region, we understand this study as a contribution to the diagnoses in this area, aiming to describe the clinicopathological and laboratory findings in six cases of ophidism in horses in Castanhal, state of Pará.

MATERIALS AND METHODS

Ethical approval. The equids in this study were patients at the Veterinary Hospital of the "Universidade Federal do Pará" (UFPA), in the Production Animal Sector. The management of these cases was not altered by the study, and ethical approval was not required. Owners signed a consent form allowing hospitalization and treatment, as well as anonymized necropsies and the use of *post mortem* samples for research purposes. The authors confirm that the study adhered to established ethical standards for animal research.

Six cases of snakebite in horses were studied: Five horses, two males and three females ranging in age from six months to eight years, and one five-year-old female mule, belonging to five properties, three of which were located in the municipalities of Castanhal and two in Rondon do Pará, state of Pará, Brazil. Medical history was obtained during visits to the properties where the cases occurred. These visits occurred sporadically, as requested by the farm owner to the veterinarians of the Veterinary Hospital of the UFPA. At that time, the clinical examination of the animals was performed according to Feitosa (2014) by the veterinary team that provided the care. In one case, it was possible to identify the snake on the property. In contrast, in the remaining cases, the veterinarians established the diagnosis of snakebite based on history, clinical signs, clinicopathological findings, and the exclusion of differential diagnoses.

Four animals (Equine 2-5) were treated with a single dose of intravenous polyvalent antivenom Master Soro Plus[®], using a 20ml ampoule, corresponding to the neutralization of 100mg of *Bothrops jararaca* venom, 100mg of *Lachesis muta* venom and 30mg of *Crotalus durissus terrificus* venom, as indicated by the manufacturer. The foal (Equine 2) received the medication between 6 and 8 hours and the other horses (3-5) between 18 and 24 hours after the bite. In addition, Equine 5 received dexamethasone 0.05mg/kg/day IM (flunixin meglumine 0.25mg/kg/ IM every 8 hours and ceftiofur 2.2mg/kg/ IM every 24 hours), in addition to hydration with 0.9% saline, 5% glucose, and lactate Ringer solution for five days. Equine 2 also received anti-inflammatory treatment and fluid therapy.

In Equine 4, an EDTA blood sample was collected to obtain a blood count and coagulogram, and in Equine 5, globular volume and coagulogram. Necropsy was performed in Equines 4 and 5, and fragments of various organs and tissues were collected and

conditioned in 10% formaldehyde. The collected material was sent for histological examination, routinely processed, and stained with hematoxylin-eosin (HE) in the “Setor de Anatomia Patológica” (Department of Pathological Anatomy) of the “Universidade Federal Rural do Rio de Janeiro” (UFRRJ) and in the “Departamento de Patologia” (Department of Pathology) of the “Universidade Federal do Rio Grande do Sul” (UFRGS).

RESULTS

Animals and epidemiological data

Primary data on race, age, sex, bite site, evolution, and outcome are shown in Table 1. Of the six animals treated, five horses and one mule (5/6) died (83% lethality). On history taking, the owners or horse caretakers reported that all equines were healthy the day before they were found ill; notable features were increased limbs or head volume, sensitivity to touch, and apathy.

Five animals (Equine 3, 4, 5, 6, and Muar 1) were bitten in paddocks close to the stables, which had *Urochloa humidicola* and *Panicum maximum cv Mombaça*. The animals drank from streams and ponds located in the paddocks. Equine 2 was bitten near the Veterinary Hospital of the UFPA, where it was staying with its mother, who was receiving medical care. On the property where Equine 4 was bitten, two snakes were found, one in the management corral with a length of 70cm and another with a size of one meter in the paddock where the animal was grazing. Both were identified as *Bothrops atrox*, also known as jararaca or northern jararaca.

Clinical signs and clinical pathology findings

On clinical examination, all horses showed apathy, loss of appetite, and increased sensitivity to touch in areas of increased volume. Three animals (3/6) were affected in the distal part of the pelvic limbs, and two (3/6) were affected in the head area, near the nostrils (Equines 2 and 5), or near the eye (Equine 6). The horses affected in the distal part of the limbs showed a marked increase in volume extending from the fetlock to the thigh. In Muar 1, the swelling spread to the vulva. These animals showed reluctance and difficulty in movement.

The horses affected in the head region (Equines 2, 5, and 6) showed an increase in volume that gave the appearance of a “rhinoceros head” (Fig.1), and the mucosa of the lips and gums had a blackened color. In Equine 2, two small holes were found near the right lip border, consistent with a snake bite. In Equine 5, the extensive head swelling prevented upper and lower lips closure and exposed the gums and oral vestibule. In this animal, the bite was noted near the nostril (Fig.2).

In addition, Equine 4 had serous bloody exudation through the intact skin at the site of swelling and bleeding around

the lower incisor teeth at the gingival margin. Equine 5 had evidence of blood exudation from the nostrils and moist cough with foam exudation (edema) from the nostrils.

All horses showed tachycardia and tachypnea, and temperature remained in the physiologic range. On pulmonary auscultation of Equine 5, moist rales were heard mainly in the cranioventral region of the right lung. The same horse that was affected by the bite in the region of the right nostril showed a marked increase in volume that extended through the nostril, mandibular, and maxillary regions into the pharyngeal region and was more pronounced on the side of the bite (right), culminating in severe inspiratory dyspnea with wheezing.

In Equine 4, the hematologic picture showed leukocytosis due to left-shifted neutrophilia and mild thrombocytosis. Equine 5 showed an abrupt drop in globular volume from 39% on the first day postbite to 15% on the sixth day of evolution. In both animals, the clotting time was more than 15 minutes. Regarding the biochemical profile, Equines 4 and 5 showed an increase in liver and kidney enzymes, as shown in Table 2. Serum total protein values remained unchanged.

Four horses (Equines 3-6) and Muar 1 died between two and seven days after the bite. Equines 3 and 4 died two days after the bite without regression of clinical signs, as did Equine 6 and Muar 1, which received no treatment. Equine 5 died on the seventh day, although clinical signs regressed. In contrast, in Equine 2, clinical signs improved from the second day after treatment, and the picture evolved toward healing.



Fig.1-2. Horse 5. (1) Enlarged head, giving it the appearance of a “rhinoceros head.” (2) Erosion sites and hemorrhages on the nasal mucosa, the bite site (arrows).

Table 1. Synthesis of the data on snakebites in the six horses

Animal	Breed	Age	Sex	Bite site	Evolution	Outcome
01	Mule	6-year-old	Female	Left pelvic limb	Without data	Death
02	Crossbreed	6-months-old	Female	Above the right nostril	Three days	Recovery
03	Mangalarga Paulista	5-years-old	Female	Right pelvic limb	Two days	Death
04	Crossbreed pony and Marajoara horse	6-years-old	Female	Left pelvic limb	36 hours	Death
05	Quarter horse	8-years-old	Male	Nostrils	Eight days	Death
06	Crossbreed	6-years-old	Male	Right upper lid	Two days	Death

Post mortem and histopathological findings

In Equine 4, necropsy revealed extensive hemorrhage in the subcutaneous tissue of the left pelvic limb with nonclotted blood, much of which was accompanied by edema (hemorrhagic edema) extending from the region of the fetlock to the midhigh (Fig.3). A large amount of noncoagulable bloody fluid was found in the abdominal and thoracic cavities (Fig.4). The kidneys had a surface with lighter areas. In addition, the diaphragm and serosa of the small intestine were severely congested.

In Equine 5, hemorrhage was conspicuous in the subcutaneous tissues of the head, mainly on the right side, extending into the submandibular region and the sulcus coronarius. Segments of the small intestine showed petechiae and ecchymosis. There were petechiae on the pleura. The lungs were pale, and there was a large amount of foam in the trachea and bronchi. There was perirenal edema, and the cut surface of the kidneys showed red streaks in the cortex and medulla, with blood clots obstructing the vessels. In addition, petechiae and ecchymosis were seen in the bladder.

Necropsy of Equine 6 revealed hemorrhagic areas on the head, chest, and right pelvic limb. The eyelid, where the lesion

was present, was identified as the site of greatest hemorrhage, indicating the location of the snakebite.

In Equine 4, histopathologic examination revealed necrosis and muscle degeneration with marked and focally extensive hemorrhage. The kidneys showed acute tubular necrosis with hemorrhage and hyaline casts. In Equine 5, the tubules in the cortical region of the kidney were frequently ectatic and filled with highly eosinophilic material (Fig.5-6). This material was also commonly seen in the urinary spaces of the glomeruli. Furthermore, multifocal deposits of basophilic and refractive material (crystals) were noted within the renal tubules. In the interstitium, there was a discrete multifocal inflammatory infiltrate of lymphocytes and plasmocytes and a discrete multifocal hemorrhage.

In the stroma of the nasal cavity, which sometimes extended to the adjacent musculature, there were multifocal to coalescent areas with marked hemorrhage (Fig.7). In the lungs, marked multifocal deposits of eosinophilic material were present in the alveolar spaces (pulmonary edema) (Fig.8), and discrete amounts of neutrophils were seen in the blood vessels and capillaries (leukocytostasis). In the liver, there was diffuse, discrete macrovacuolar degeneration of hepatocytes and a discrete multifocal inflammatory infiltrate of lymphocytes and plasmocytes.

Table 2. Biochemical findings of bothropic envenomation in horses, Castanhal, Pará, Brazil

Tests	Horse 4	Horse 5	Reference values*
AST (U/L)	291	552	226 - 366
GGT (U/L)	28,3	3,8	4.3 - 13.4
Urea (mg/dL)	68	347	21 - 51
Creatinine (mg/dL)	2.1	10.5	1.2 - 1.9

* Reference Kaneko et al. (1997).

DISCUSSION

Ophidian accidents are usually related to climatic fluctuations and rainfall that cause snakes to seek dry land when riverbeds, dams, and streams overflow (Bernarde et al. 2012, Milani et al. 2016). However, with deforestation and replacement by pastures, a higher occurrence of these accidents is observed

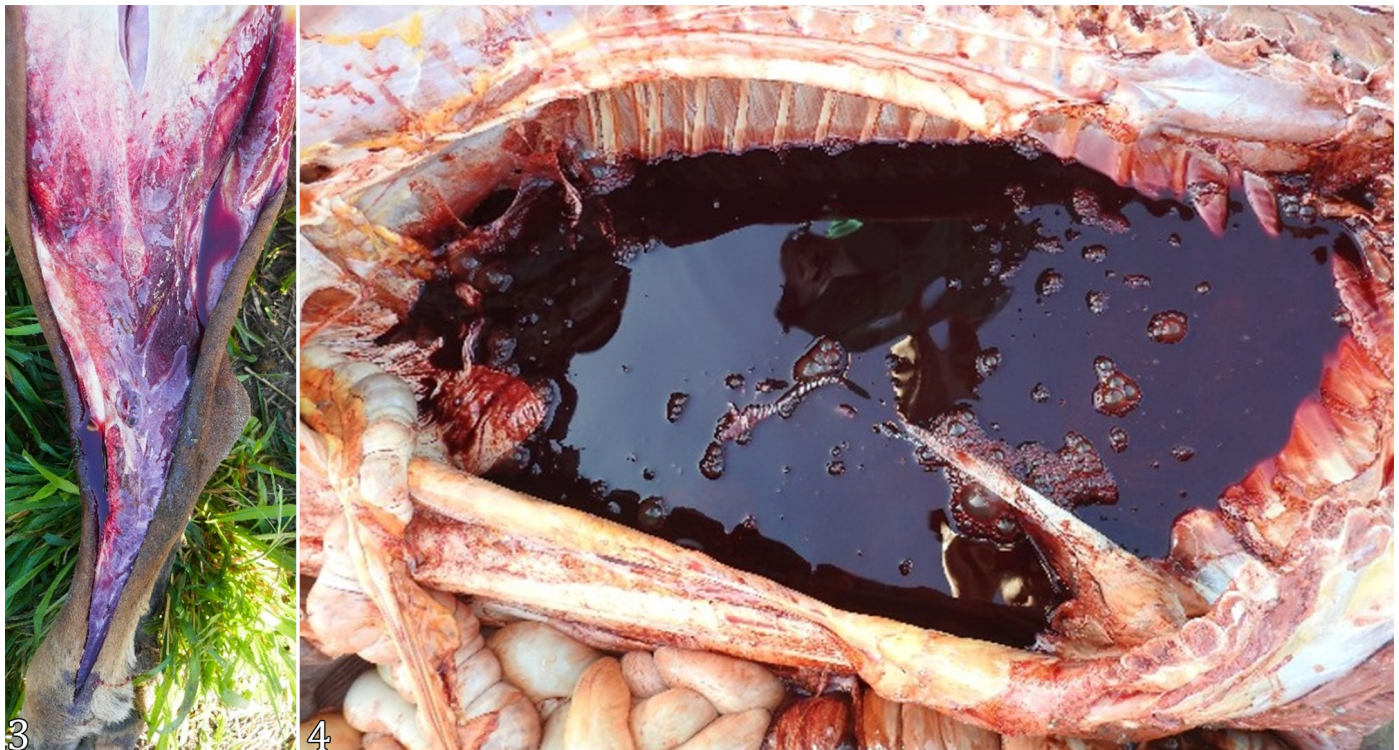


Fig.3-4. Equine 4. (3) Extensive hemorrhage in the subcutaneous tissue of the pelvic limbs, corresponding to the bite site. (4) Large amount of uncoagulable blood in the cavities.

(Fearnside 2005) due to the destruction of the natural habitat of ophidians and their migration to other areas (Bernarde & Gomes 2012). Our work highlights that five affected animals were in the circumstances described above.

The diagnosis of snakebite in the horses of the present study by snakes of the genus *Bothrops* was based on the history, clinicopathological findings and exclusion of differential diagnoses, which was reinforced by the presence of *Bothrops atrox* snakes (jararaca, northern pit viper) on the property where Equine 4 lived. In Brazil, accidental poisonings in horses by *Bothrops* spp. have been reported in São Paulo (Cano et al. 2016, Camplesi et al. 2017), in Rio Grande do Sul (Raposo et al. 2001, Pierezan et al. 2009), in Paraná (Bello & Malschitzky 2021), in Pernambuco (Tolentino et al. 2019), in the Midwest

(Machado et al. 2019), and in Pará (Silva et al. 2011) have been reported. However, the significance of this study is not limited to the description of other snakebites in Pará; it also highlights the first report of a snakebite on a mule in Brazil.

Although fatal cases of ophidism in livestock are considered overestimated (Tokarnia & Peixoto 2006), the present study reports the death of 83% of animals affected by *Bothrops* envenomation (5/6), which is considered a high lethality. According to Tokarnia & Peixoto (2006), the relationship between the dose of injected venom and the animal's weight would be a factor in animal lethality. However, in this study, fatal cases are described in five adult animals weighing between 250 and 500kg, while the other animal, a 60kg, 6-month-old foal, recovered completely.

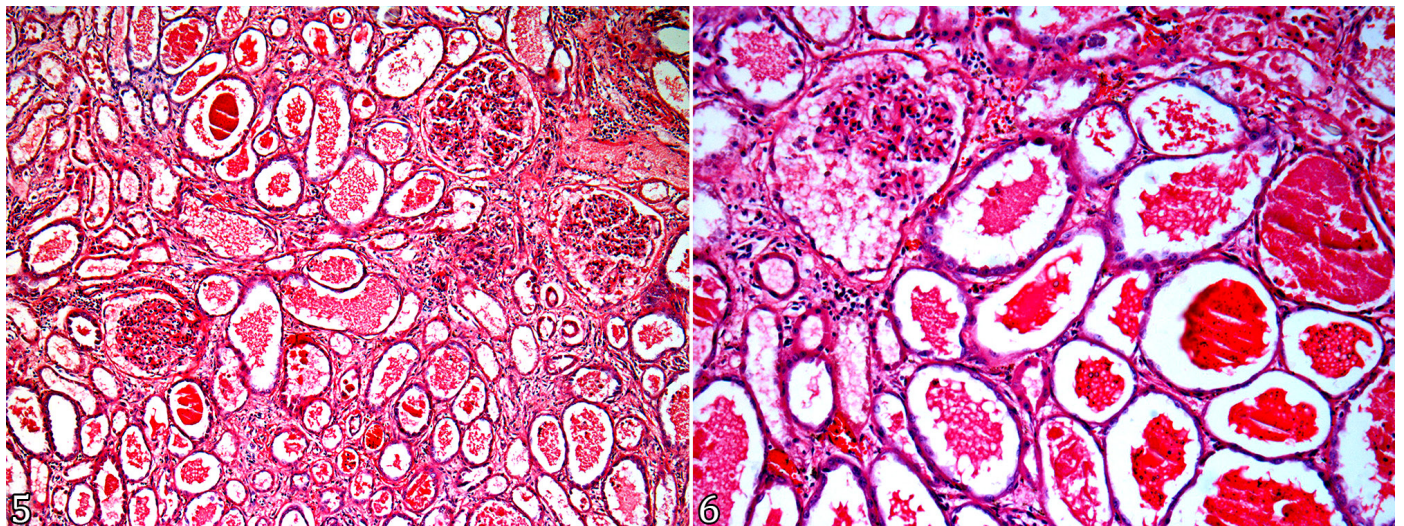


Fig.5-6. Equine 5. Kidney. Increased glomerular filtrate (strongly eosinophilic material) also fills the lumen of the renal tubules, most of which are ectatic. (5) HE, obj.10x. (6) HE, obj.20x.

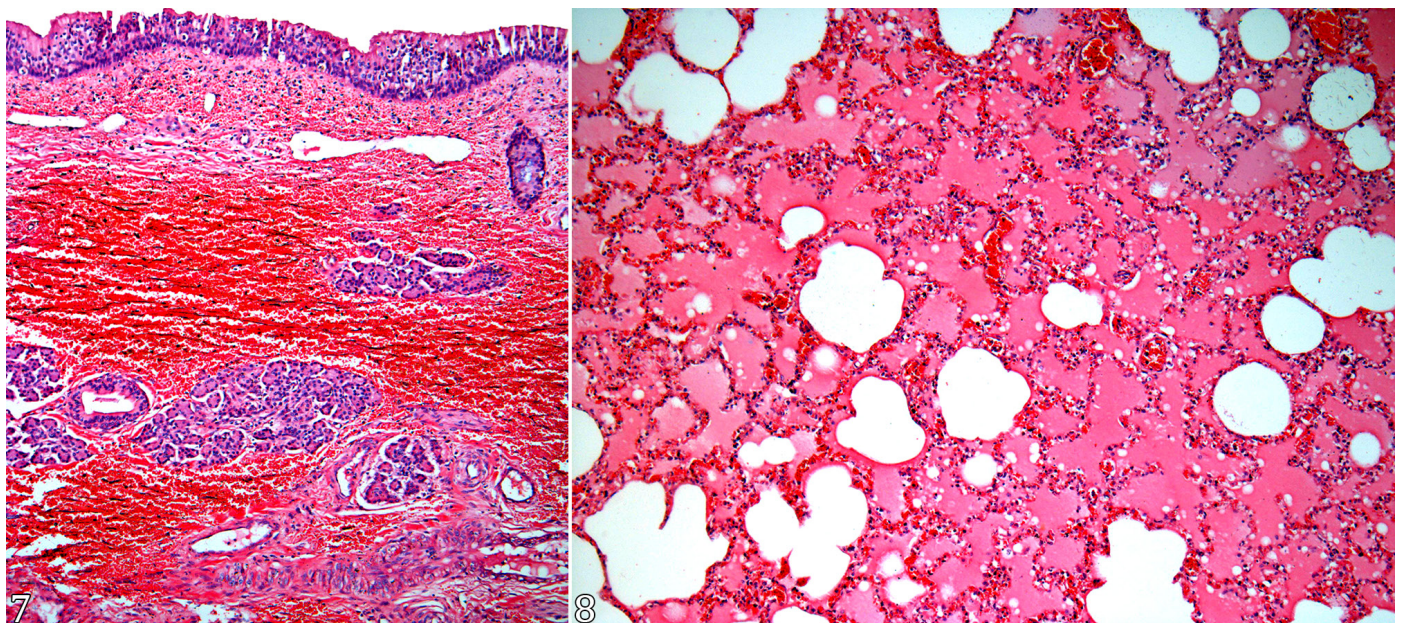


Fig.7-8. Equine 5. (7) Nasal cavity. Multifocal to coalescent areas of marked hemorrhage. HE, obj.10x. (8) Lung. Marked multifocal deposition of amorphous eosinophilic material in the alveolar spaces (pulmonary edema). HE, obj.10x.

The fact that the younger horse recovered, which theoretically would not have been expected, indicates the importance of other variables, such as the susceptibility of the animals, the location reached, the amount of venom inoculated, the escape of the animal during the attack of the snake, the escape of the snake before the injection of all the venom, the age of the snake, state of satiety of the snake, the timing of the start of treatment, and other variables (Tokarnia & Peixoto 2006, Santos et al. 2016) that have not been clarified. Although only one case of ophidism in a mule is described in this study, it is noted that the animal was sensitive to poisoning, like horses.

The location and amount of poison introduced influence the severity of the case. In affected animals, especially in the head area, injuries may cause compression of the respiratory tract due to edema and hemorrhage, aggravating the condition. Curious behavior and the habit of grazing justify the injuries in the head area, and the limbs and abdomen are frequently bitten (Grunert & Grunert 1969, Sousa et al. 2011). In the present study, three animals (3/6) were hit in the head area, and one of them (Equine 5), which was bitten in the nostril, had a severe respiratory condition; both were in pickets. The target circumstances for the occurrence of the accident (exposure in a paddock and location of the bite) favored the event.

The most pronounced clinical sign of a *Bothrops* accident is the voluminous hematoma near the bite site resulting from the inflammatory process initiated by the venom (Grunert & Grunert 1969). Other signs, such as pain, tachycardia, and tachypnea, are also commonly described and highlighted in both experimental cases and cases of natural poisoning (Tokarnia & Peixoto 2006, Sousa et al. 2011, Ferreira et al. 2020, Machado et al. 2019, Tolentino et al. 2019). In addition to these changes, the animals in this work also showed apathy, loss of appetite, and difficulty in locomotion.

As for the laboratory results, the anemia of Equine 5 was due to the hemorrhages caused by the action of the venom. The leukocytosis due to left-shifted neutrophilia developed in Equine 4 is compatible with the inflammatory state described in snakebites, which promotes the recruitment of leukocytes and the consequent increase in blood flow (Rucavado et al. 2016). The nephrotoxic effect of bothropic venom justifies the increase in serum levels of urea and creatinine observed in Equines 4 and 5; this finding has also been described previously (Machado et al. 2019).

Although four animals were treated, the foal (Equine 2) was believed to be the only animal that survived due to the rapid diagnosis and treatment as it was located at the UFPA Veterinary Hospital, preventing the clinical picture from worsening.

CONCLUSION

This case report demonstrates the clinical and laboratory manifestations of Bothropic envenomation in horses, characterized by marked volume increase, extensive hemorrhage, and systemic changes. Despite the recovery of the foal described in this case, the findings highlight the need for further research into the pathophysiology of snakebites in livestock to better understand the factors involved in the progression of fatal cases. In addition, this report seems to be the first documentation of bothropic envenomation in a mule in Brazil that showed a clinical course similar to that of other horses.

Conflict of interest statement.- The authors declare that there are no conflicts of interest.

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