Ciência Rural, Santa Maria, v.50:5, e20190940, 2020 http://dx.doi.org/10.1590/0103-8478cr20190940
ISSNe 1678-4596
PATHOLOGY

Acute renal failure in a horse following bee sting toxicity

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ABSTRACT: *Bee envenomation is frequent in humans and dogs, but uncommon in horses. This study aimed to describe a case of acute renal failure following mass envenomation in a horse. A mare was attacked by a swarm of bees and showed reluctance to move, epistaxis, and dark-brown urine. Biochemical exams revealed increase in urea and creatine serum levels. The mare did not respond to treatment and euthanasia was elected after four days of clinical course. At the necropsy, there were multifocal pinpoint to elevated skin lesions associated with edema and hemorrhage, which extended to the subcutaneous tissue and skeletal muscle, and the kidneys were diffusely dark-brown and friable. Microscopically, renal tubules were distended and filled with an orange-red, hyaline globular material, and had severe epithelial tubular cell necrosis. The diagnosis was established based on clinical and histological analysis, and pathological evaluation was essential to confirm acute renal failure due to bee sting toxicity.*
Key words: *acute renal failure envenomation, pathology, veterinary toxicology.*

Insuficiência renal aguda associada à intoxicação por picadas de abelhas em um cavalo

RESUMO: *Acidentes por picada de abelhas são frequentemente descritos em humanos e cães, entretanto relatos em cavalos são escassos. Este trabalho teve como objetivo descrever um caso de insuficiência renal aguda em um equino após múltiplas picadas de abelhas. Uma égua foi atacada por um enxame de abelhas desenvolvendo quadro clínico de relutância em se movimentar, epistaxe e urina marrom-escura. Exames bioquímicos demonstraram aumento nos níveis séricos de ureia e creatina. O equino não respondeu ao tratamento e a eutanásia foi realizada após quatro dias de curso clínico. Na necropsia havia múltiplas elevações cutâneas, que ao corte exibiam edema e hemorragia, os quais se estendiam ao subcutâneo e musculatura adjacente. Os rins estavam difusamente marrom-escuros e havia friáveis. Microscopicamente, os túbulos renais estavam distendidos e preenchidos por um material hialino, globular, laranja-avermelhado e havia acentuada necrose das células epiteliais tubulares. O diagnóstico foi obtido por meio da análise clínica e histológica, e a avaliação patológica foi essencial para confirmar a insuficiência renal aguda por picada de abelha.*
Palavras-chave: *insuficiência renal aguda, intoxicação, patologia, toxicologia veterinária.*

African honey bees (*Apis mellifera scutellata*) were introduced in Brazil in the 1950s, and uncontrollable cross-breeding with European bees (*Apis mellifera mellifera*) occurred, resulting in an extremely aggressive hybrid Africanized bee. The bee venom is composed mainly by melittin and phospholipase A2, which are responsible for cell membrane damage and allergenic responses (FITZGERALD & FLOOD, 2006). The response to the bee venom may occur as a local or regional reaction, systemic reaction or anaphylaxis, delayed reaction, and as a massive envenomation or envenoming syndrome (HUGHES, 2019). Anaphylaxis is associated with most deaths; however, there are fatal cases due to envenomation related to multiple stings (FITZGERALD & FLOOD, 2006, HUGHES, 2019). There are several reports of bee envenomation in humans (HUGHES, 2019) and in dogs (OLIVEIRA et al., 2007); however, cases in horses have been focused on the clinical rather than the pathological features (STAEMPFLI et al., 1993; LEWIS & RACKLYEFT, 2014; FONTEQUE et al., 2018; VEADO et al., 2020). Thus, this study aimed to describe the clinical and pathological findings of

Received 11.28.19 Approved 01.30.20 Returned by the author 03.23.20
CR-2019-0940.R1

a fatal case of acute renal failure in a horse after a massive bee envenomation.

A four-year-old Criollo mare was attacked by a swarm of Africanized bees, and showed reluctance to move (suggestive of severe muscular pain), epistaxis, and dark-brown urine in a four-day clinical course. Complete blood count (CBC) and partial serum biochemical tests were performed. Laboratory analysis revealed an increase in urea [28.64 mmol/L, reference value (RV): 4-9 mmol/L] and creatine serum levels (793.83 µmol/L, RV:106.08-159.12 µmol/L). The CBC revealed mild leukocytosis (14.8G/L, RV: 5.8-13.2 G/L), while the other parameters were unremarkable. The horse was treated with intramuscular administration of 0.1 mg/kg of dexamethasone once a day, and supportive treatment with fluid therapy, but, since no clinical response was observed, euthanasia was obtained through intravenous injection of barbiturates, followed by an infusion of potassium chloride, according to the official guidelines (CFMV, 2012).

At the necropsy, the oral, ocular, and vulvar mucosae were severely cyanotic, and large amounts of foamy liquid and blood filled both nostrils (Figure 1A). The skin of the head and limbs had multiple pinpoint to elevated lesions, ranging from 0.5 to 1.0 cm in diameter, which were associated with moderate edema and hemorrhages in the subcutaneous tissue, often extending into the skeletal muscles (Figure 1B). The abdominal cavity contained four liters of serous light yellow liquid (ascites). The perirenal fat tissue had a diffuse gelatinous aspect (severe edema), and the kidneys were markedly dark-brown and friable (Figure 1C). On the cut surface, the renal cortex was finely mottled (Figure 1D). Moreover, the urinary bladder had a small amount of red urine intermixed with blood clots, while the mucosa was reddened with multifocal petechial to ecchymotic hemorrhages. The lungs were enlarged, dark-red and did not collapse at the opening of the thoracic cavity. Large amounts of foamy liquid filled the upper and lower airways, and on the cut surface the lungs exuded blood. Multiple samples of organs were collected, fixed in 10% neutral buffered formalin, routinely processed for histology, and stained by hematoxylin and eosin (HE).

Histological examination of the kidneys revealed diffuse and marked distension of proximal and distal convoluted tubules by an orange-red, globular, hyaline material (suggestive of myoglobin; Figure 1E). These were associated with moderate tubular epithelial cell necrosis, which was characterized by attenuated epithelium with flattened cytoplasm, sloughing tubular cells with hypereosinophilic cytoplasm and pyknotic nuclei (Figure 1F). The skeletal muscle had multifocal areas of hemorrhage, associated with mild myofiber necrosis, which was characterized by swollen cells with hypereosinophilic, homogenous cytoplasm, which frequently lacked cross striations. Moreover, there were disseminated hemorrhages in multiple organs, such as the lungs, the adrenal glands, and the stomach, besides diffuse congestion involving the lymph nodes and lungs, which also showed moderate and diffuse alveolar edema.

In the present study, the diagnosis of acute renal failure due to bee sting toxicity was made through the association of the clinical and histological findings. Although commonly described in humans (HUGHES, 2019) and dogs (OLIVEIRA et al., 2007), case reports on bee sting toxicity in horses are scarce and mainly focused on the clinical findings (STAEMPFLI et al., 1993; LEWIS & RACKLYEFT, 2014; FONTEQUE et al., 2018; VEADO et al., 2020). Bee envenomation may cause a variety of clinical presentations, including cutaneous lesions, local swelling, muscular pain, anaphylactic reaction, cardiovascular collapse, hemolysis, rhabdomyolysis, multiorgan damage, and acute renal failure (HUGHES, 2009). In the present case, the mare had multiple skin lesions, characterized by raised areas associated with local swelling and hemorrhage. This is also a common finding in dogs (OLIVEIRA et al., 2007), humans (HUGHES, 2019) and horses (STAEMPFLI et al., 1993; VEADO et al., 2020), and it is most likely caused by direct effect of melittin and phospholipase A_2, which leads to endothelial cell membrane injury (LEWIS & RACKLYEFT, 2014).

Furthermore, increased urea and creatinine blood levels suggested acute renal failure in this case, which was later confirmed through the gross and microscopic pathological changes. These findings are similar to the ones described in massive bee attacks in humans (HUGHES, 2019), dogs (MUGHAL et al., 2014) and horses (LEWIS & RACKLYEFT, 2014), and the pathogenesis of this lesion was most likely related to direct nephrotoxicity and hypotension due to the venom vasoactive components (FITZGERALD & FLOOD, 2006). The reduced perfusion of the renal tissue may have reduced the glomerular filtration rate and, consequently, caused urine stasis at the proximal and distal tubules of the kidney, leading to tubular epithelial necrosis (CIANCIOLO & MOHR, 2016). This presentation is similar to what has been previously described in mass envenomation in horses (STAEMPFLI et al., 1993; LEWIS & RACKLYEFT,

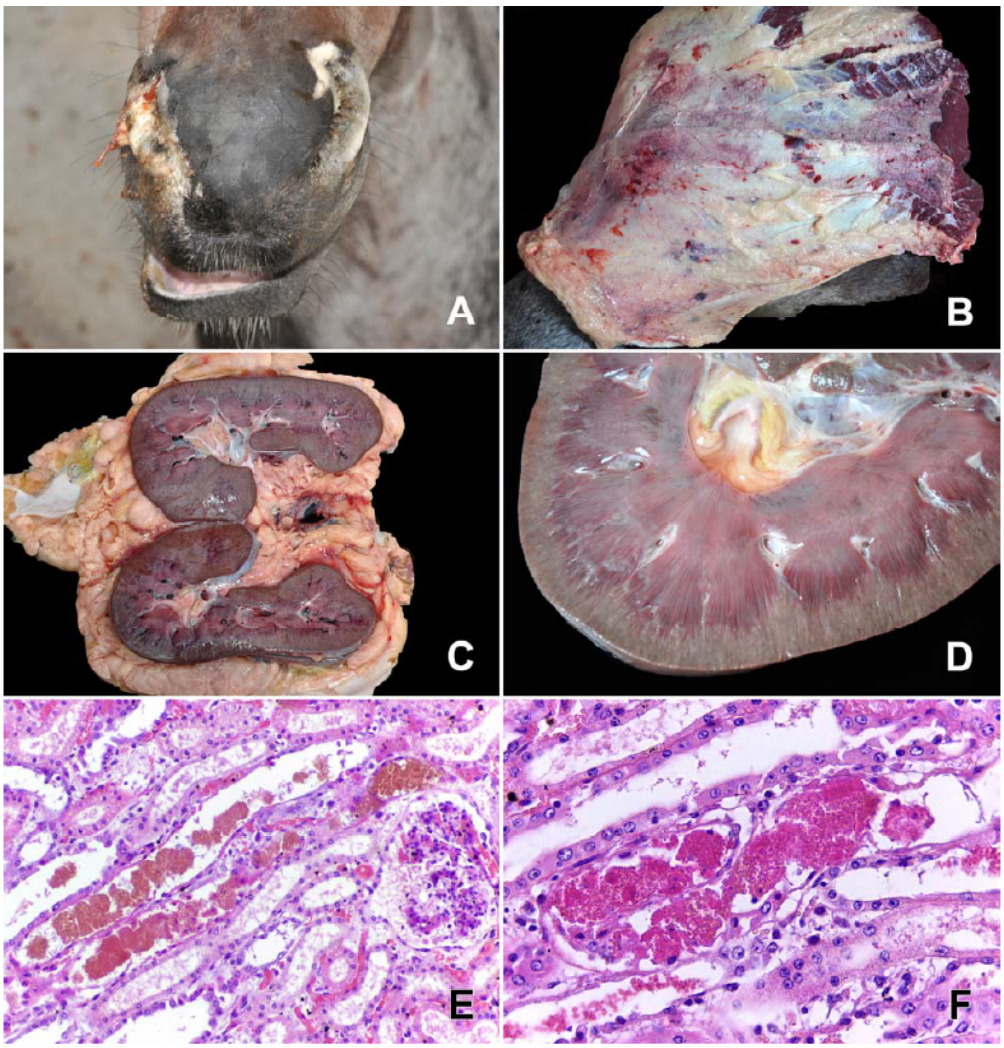

Figure 1 - Acute renal failure in a horse following bee sting toxicity. (A) Both nostrils were filled with large amounts of foamy liquid and blood. (B) Multiple hemorrhages at the subcutaneous tissue, extending into the skeletal muscle. (C) The kidneys were markedly dark-brown and the perirenal fat tissue showed severe edema. (D) On the cut surface, the renal cortex was finely mottled. (E) The renal tubules were severely distended and filled with an orange-red, globular, hyaline material (myoglobin). Hematoxylin and eosin (HE), 20x. (F) This was associated with epithelial tubular cell necrosis, characterized by attenuated epithelium and sloughed cells with hypereosinophilic cytoplasm and pyknotic nuclei. HE, 40x.

2014; FONTEQUE et al., 2018). This hypothesis was supported by the histological analysis of the kidney, which revealed multiple intratubular hyaline casts intermixed by necrotic epithelial tubular cells. These lesions probably resulted in the gross changes of dark brown and friable kidneys, which are consistent with other fatal cases of bee sting envenomation in dogs (OLIVEIRA et al., 2007).

Moreover, a hematological change noticed in this case was mild leukocytosis, which may be related to tissue damage and inflammation induced by bee stings in horses (LEWIS & RACKLYEFT, 2014; FONTEQUE et al., 2018), but in the present case it could be related to dexamethasone treatment, since no inflammation was histologically detected in the skin. In addition, the mare presented bilateral

epistaxis, and at the necropsy disseminated hemorrhages and congestion involved multiple organs. This has been previously observed in dogs (OLIVEIRA et al., 2007) and in horses (STAEMPFLI et al., 1993; LEWIS & RACKLYEFT, 2014), and it is most likely a result of coagulopathy induced by phospholipase A_2 (OLIVEIRA et al., 2007). Dark red, wet and enlarged lungs induced by lung edema were previously described in cases of massive bee attack in a horse (STAEMPFLI et al., 1993), dogs (OLIVEIRA et al., 2007), and sheep (VEADO et al., 2020), as it was observed in the present case. Still, this mare had severe pulmonary edema, which extended into the upper airways and through the nostrils, differing from previous reports of necropsy in animals affected by bee stings (STAEMPFLI et al., 1993; OLIVEIRA et al., 2007).

Differential diagnosis should include other insects causing similar lesions, such as wasps (FITZGERALD & FLOOD, 2006), and other causes of acute renal failure, such as hemodynamic alterations, which were excluded based on the clinical and pathological findings, and nephrotoxic drugs (CIANCIOLO & MOHR, 2016), which were not previously employed in this case. Other myopathies are common in horses and should be considered, such as ionophore toxicosis, exertional rhabdomyolysis, and polysaccharide storage myopathy. The latter two were excluded due to the history of the mare, as well as due to the type of muscle lesions, which were characterized by monophasic necrotic and hemorrhagic mild lesions, in which no glycogen vacuoles and inclusions involved the myofibers, as may occur in polysaccharide storage myopathy (COOPER & VALENTINE, 2016). Moreover, multiple random areas of skeletal muscles were affected, differing from widespread lesions related to exertional rhabdomyolysis and mainly myocardial damage in ionophore toxicosis. Likewise, multiorgan damage, as it was observed in this case, is not described in these myopathies (COOPER & VALENTINE, 2016). The clinical and histological findings were essential to obtain the final diagnosis in this case, associating the history of attack by a swarm of bees with the clinical signs and the pathological features.

ACKNOWLEDGEMENTS

This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and it was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). Brasil - Finance code 001.

BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

We authors of the article entitled “Acute renal failure in a horse following bee sting toxicity” declared, for all due purposes, the project that gave rise to the present data of the same has not been submitted for evaluation of the Ethics Committee of the Universidade Federal do Rio Grande do Sul (UFRGS), but we are aware of the content of the Brazilian resolutions of the Conselho Nacional de Controle de Experimentação Animal (CONCEA) if it involves animals. Thus, the authors assume full responsibility for the presented data and are available for possible questions, should they be required by the competent authorities.

DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The funding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS’ CONTRIBUTIONS

All authors contributed equally for the conception and writing of the manuscript. All authors critically revised the manuscript and approved the final version.

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