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CASE REPORT
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Toxic Systemic Reaction after Bee Stings in a Bitch

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ABSTRACT

***Background*:** Bee sting poisonings are common in dogs, and toxic systemic presentation may represent a life-threatening condition. *Apis mellifera* venom is a complex mixture of melitin, apamine, phospholipase, hyaluronidase and degranulating peptides, that causes local injury at the site of inoculation and multiple organ complications, including hemolysis, kidney injury, muscular damage, cardiovascular and respiratory complications. The present work reports a complete and detailed description of a dog's systemic toxic reaction to bee stings, including history, clinical signs, laboratory findings, emergency care and development, as well as possible association with later immunomediated arthritis.

***Case*:** A 6-year-old female German Shepperd suffered multiple bee stings. First care was conducted by a veterinary at the site, where he only received promethazine, meloxicam and dexamethasone. After 24 h and significant progression of symptoms, the animal was forwarded to a specialized veterinary hospital. The patient was evaluated throughout 9 days, and presented intense edema, respiratory distress, tongue necrosis and grade II of acute kidney injury. Extensive laboratory exams were conducted throughout the hospitalization. Main laboratory findings included polycythemia, leukocytosis by neutrophilia and monocytosis, thrombocytopenia and azotemia. Urinalysis evidenced turbid aspect, dark yellow color and intense proteinuria, reinforcing kidney damage. Abdominal ultrasound examination identified blood clots in the bladder, and liver with reduced echogenicity and echotexture, suggesting acute inflammation. Therapy aimed to stabilize the patient, control kidney damage and avoid anaphylaxis. Treatment included intensive care support, promethazine, hydrocortisone, dexamethasone, dipyrone, methadone, metronidazole, ampicillin, clindamycin and tramadol. Following successful treatment, the animal presented immunomediated polyarthritis, possibly associated to both the poisoning and later diagnosed hemoparasitosis (both *Erlichia* and *Babesia*).

***Discussion*:** Massive bee attacks can cause severe complications, however, data regarding emergency care records are scarce. Based on clinical signs and laboratory findings, the patient presented toxic systemic reaction, including grade II of acute kidney injury and significant cardiorespiratory distress. Another important complication was tongue necrosis, that demanded attention and special supportive care, including feeding tube and specific feed. Treatment also focused in reducing edema and control possible anaphylaxis, providing analgesia and antibiotic therapy. Laboratory findings have been previously described, with evidence of immune-mediated reaction. Follow-up consultations revealed normal parameters, and an unusual presentation of claudication. Investigation concluded that polyarthritis could be responsible for such finding and may be a result of the deposition of immunomediated complexes in the joints, due in this case to the bee poisoning and later positive diagnosis for both *Erlichia* and *Babesia*. Systemic reactions to bee stings are complex, and full clinical and laboratory profile aid in both the prognosis and treatment options. Special attention must be given to tongue damage and supportive care is essential for maintaining feeding conditions. Arthritis should be considered as possible complication, reinforcing the importance of follow-up consultations.

Keywords: bee attack, dog, canine, envenomation, melittin, poisoning, phospholipase A2.

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INTRODUCTION

Accidents caused by bee stings have become a public health concern [18], especially after the introduction of "Africanized bees" in the American continent. In Brazil, an *Apis mellifera* species, popularly known as the Africanized bee, arose from a cross between European bees *(Apis mellifera* and *Apis linguistica*) and African bees (*Apis mellifera scutellata*) [6]. In addition to the high number of stings, the persistence in continuing to attack the enemy is also typical [2].

Bee stings can cause medical emergencies [18] due to its venom, composed of a complex mixture of biochemically active substances, including mellitin, phospholipase A2 [2,8,21], hyaluronidase, apamine and degranulator peptide [10]. Clinical presentation after multiple bee stings includes hypersensitivity and systemic toxicity [22]. The latter includes damage to the kidneys [10], liver, and muscles, causing apathy, vomiting, diarrhea, respiratory distress, acute renal failure, and shock [1,7,23].

The incidence of accidents by bees is unknown in Veterinary Medicine due to the absence of records, or the absence of specialized care in mild to moderate cases. The present case report describes a toxic systemic reaction to bee stings in a bitch, followed by an unusual immune mediated arthritis.

CASE

A 6-year-old bitch German Shepperd weighing 36 kg, was presented to veterinary assistance at the São Francisco de Assis Veterinary Hospital (HVSFA) in Belo Horizonte, MG, Brazil, with a history of having suffered an attack by a swarm of bees 24 h prior. During anamnesis, the tutors reported that after the attack, the animal was assisted by a home care veterinary, that prescribed promethazine, meloxicam and dexamethasone (doses unknown). However, the following day, the patient's condition worsened, presenting prostration, loss of appetite, diarrhea and muscle tremors, and, therefore, she was brought to the HVSFA care.

At the physical examination, the animal presented dehydration of 10%, congested mucosa, rectal temperature of 40°C, heart rate (HR) of 240 beats per min (bpm), tachypnea, oxygen saturation in 91%, and blood glucose in 40 mg/dL. In addition, the patient had swollen tongue, filled with stings, both eyes and oral mucosa presented moderate bleeding (Figure 1), as well as bloody diarrhea and hematuria. The behavior was extremely aggressive and the patient had a vision deficit (difficulty to avoid obstacles and absence of threat reflex). Episodes of motor incoordination and dysphagia were also observed.

Due to the seriousness of the case, the patient was admitted to a semi-intensive care. Initial treatment was provided with nasal tube placement to provide oxygen support, promethazine[1] [1 mg/kg, Intravenous (IV)], hydrocortisone[2] [10 mg/kg, IV], dexamethasone[2] [0.3 mg/kg, IV], and dipyrone[3] [25 mg/kg, IV] and methadone[4] [0.3 mg/kg, Subcutaneous (SC)]. The patient was placed under monitoring with an electrocardiogram at bedside, that identified sinus tachycardia. To control pain, an intravenous bolus of fentanyl[4] was given [5 mcg/kg] and then continuous infusion was started [5 mcg/kg/h]. The patient also received epinephrine[5] [0.1 mg/kg] and was placed on fluid therapy with 2.5% glucose supplementation[6].

After medications and oxygen support, HR dropped to 120 bpm and oxygen saturation reached 94%. Patient underwent a urethral probe application to assess urinary output. Urinary output, calculated at the end of the day, was 0.7 mL/kg/day, and urine had intense red color. Therefore, furosemide[3] [2 mg/kg, IV] was added. Following stabilization, large numbers stings were removed from the face and tongue.

On the 1st day of admission (day 1), a complete blood count and biochemical profile were requested. The blood count showed polycythemia (8.83 millions/mm^3; reference of 5.5 - 8.5 millions/mm^3), leukocytosis (21,200/mm^3; reference of 6,000 - 17,000/mm^3) by monocytosis (65,000/mm^3; 150 - 1,250/mm^3) and lymphocytosis (13,300/mm^3; reference of 1,000 - 4,800/mm^3), and thrombocytopenia (63,000 mil/mm^3; reference of 175 - 500 mil/mm^3). Biochemical profile showed azotemia, urea of 109.14 mg/dL (reference of 21 - 102 mg/dL) and creatinine of 1.8 mg/dL (reference up to 1.4 mg/dL).

Prescription on the 1st day included promethazine[1] [1 mg/kg, IV twice a day], hydrocortisone[2] [10 mg/kg, IV, 3 times a day (TID)], ondansetron[7] [0.5 mg/kg, IV, TID] and dipyrone[3] [25 mg/kg, IV, TID]. On the 2nd day (day 2 of hospitalization), the patient was prostrate, but responding to manipulation. Numerous bites were removed with tweezers throughout the animal's body, taking care not to squeeze the stinger, which could cause greater venom release. At the bite sites, there

was moderate erythema and mild edema, except on the face, which had moderate edema. The tongue was less swollen compared to the day before, however, there were areas of necrosis (Figure 1), in addition to the oral bleeding that still persisted. Thus, the patient was anorexic and underwent a nasogastric tube insertion to receive nutritional support. Feeding was done with Premier® gastrointestinal ration[8], supplied every 4 h. In addition, the use of Hexomedin[1], TID, was instituted as a treatment for wounds in the oral cavity.

Regarding clinical parameters, general status was still regular, rectal temperature was 38.6ºC, systolic blood pressure (SBP) was 112 mmHg, blood glucose was 199 mg/dL and urinary output was 1.5 mL/kg/h. There were 2 episodes of bloody diarrhea and one episode of vomiting on this day (48 h after attack). The vomit, in addition to food content, contained bees. The patient was still receiving oxygen support via a nasal tube.

During the entire hospital stay (total of 9 days), hematological and biochemical follow-up examinations were performed. On 2nd day, a new blood count, urinalysis, urinary protein/creatinine ratio and blood gas analysis. Blood count showed an even greater leukocytosis than the previous exam (133,300/mm^3), with intense neutrophilia (109,306/mm^3; reference of 300 - 11,500/mm^3) and thrombocytopenia (39,000 mil/mm^3). Urinalysis evidenced turbid aspect, dark yellow color, and 10 blood cells/field. Urinary protein/creatinine relation was 3.12 (142 protein, and 45.4 of creatinine), indicating intense proteinuria. Blood gas revealed discrete acidosis (pH of 7.34; reference of 7.36 - 7.44), with high PCO_2 (46.3 mmHg; reference of 35.0 - 38.0 mmHg) and high HCO_3 (25.1 mEq/L; reference of 15-23 mEq/L).

Antimicrobial therapy was added to the medical prescription: metronidazole[9] [15 mg/kg - twice a day (BID] and ampicillin[10] [20 mg/kg, TID], in addition to a single dose IV of clindamycin[11]. For analgesia due to muscular pain, tramadol[3] [2 mg/kg, TID, SC], ketamine[12] [0.5 mg/kg, QID, IV], scopolamine butylbromide[13] [25 mg/kg, TID, IV], and gabapentin[14] [10 mg/kg, SID, oral dose (VO)] were prescribed. Sucralfate[14] [1 g/animal, TID, VO] was also administered.

The patient had another episode of oliguria between the night of the 2nd and 3rd day of hospitalization, with a urinary output measurement of 0.6 mL/kg/day. Two new administrations of furosemide [2 mg/kg, IV] were made, 4 h apart. In addition, the fluid therapy rate was increased to 150 mL/kg/h for 24 h. Oxygen supply was gradually withdrawn, until the patient was stable without support. Hematological exams and the dosage of urea and creatinine were performed again. Most significant findings were leukocytosis of 135,800/mm^3, due to neutrophilia (88,270/mm^3), monocytosis (5,432/mm^3) and lymphocytosis (31,234/mm^3), with significant reduction of platelets (39,000/mm^3). Azotemia continued with urea of 108 mg/dL and creatinine of 1.59.

On the 4th day of hospitalization, hematological tests were repeated, showing normalization of hematological and leukocyte parameters (8,600/mm^3). However, low platelets remained (43,000/mm^3). Fluid therapy rate was decreased to 100 mL/h. Also on the 4th day, an abdominal ultrasound examination identified blood clots in the bladder, and liver with reduced echogenicity and echotexture, suggesting acute inflammation. Other organs remained normal. General condition was regular, clinical parameters were within the normal range, and she gradually started to walk and see again.

Only on the 5th day of hospitalization, the urine output was satisfactory again (1.5 mL/kg/day), and the color was closer to normal (yellowish). Hematological exams were repeated on the same day for follow-up, and the most significant finding was the platelet level, that remained low (36,000/mm^3). In addition, the dosage of alanine aminotransferase (ALT) and total bilirubin, direct and indirect was requested to assess the liver profile. Only ALT was increased (712 IU/L; reference of 21 - 102 IU/L).

On the 6th day of hospitalization, there was loss of tongue tissue where necrosis was identified (Figure 1). The animal still had some mouth bleeding. Also on the same day, a new blood gas analysis was requested, the result of which showed hypokalemia (2.6 mEq/L; reference of 3.40 - 4.90 mEq/L). Potassium replacement was performed intravenously (40 mEq; maximum rate of 0.5 mEq/kg/h).

On the following days of hospitalization, the patient showed significant clinical improvement. The bitch was alert, responsive, docile and began to feed voluntarily, still with a pasty diet. Urine was normal and straw yellow in color. On the 9th day of hospitalization, hematological tests were repeated, urea and creatinine were measured, with the aim of evaluating the patient's possible discharge. Blood analysis showed anemia,

erythrogram of 4.27 millions/mm^3, hemoglobin of 9.6 g/dL (reference of 12 - 18 g/dL) and hematocrit of 28% (37 - 55%). Platelet level remained low, at 90,000 mm^3. Urea and creatinine remained normal.

In view of these results and good general condition, the patient was discharged from hospital. The following drugs were prescribed homecare: prednisone[4] [40 mg/m^2, VO], until further recommendations; amoxicillin with potassium clavulanate[14] (25 mg/kg, BID for 7 days, VO]; tramadol[3] [4 mg/kg BID for 5 days, VO]; silymarin[15] [25 mg/kg SID, VO], until new recommendations; oral cleaning hexomedine[1] [TID, for 5 days]. In addition, the recommendation regarding nutrition was to keep the diet soft, until total improvement of lesions in the oral cavity.

The patient returned for reassessment 1 week after discharge, with a good general condition, improved tongue condition and vital parameters within normal range (Figure 1). On the skin, there were small scabs in the healing phase, as a result of the stings. On that day, hematological exams were repeated to monitor the condition, as well as the biochemical profile. Hematological examination showed mild, regenerative anemia, as well as mild neutrophilic leukocytosis. Erythrocyte at 4.42 millions/mm^3, hemoglobin of 10.4 g/dL, hematocrit of 34.6%. Discrete leukocytosis of 19,400/mm^3, due to neutrophilia 16,684/mm^3. In addition, the number of platelets returned to a value within the reference range (237,000/mm^3), and biochemical profile showed improvement in relation to liver function (ALT of 216 IU/L)and normal kidney parameters (urea and creatinine).

Prednisone[4] dose was reduced [from 40 mg/m^2 to 20 mg/m^2 SID for 7 days, followed by 5 days of 10 mg/m^2 SID, and 10 mg/m every 48 h for an additional 5 days]. One week later, the patient was brought back to the HVSFA, complaining of pain in her right pelvic limb. Clinical examination showed crepitation in the joints, both of the thoracic and pelvic limbs. Pelvis x-ray showed moderate bilateral hip dysplasia and bilateral moderate degenerative joint disease. Spinal examination showed no changes. Immunochromatographic tests for *Erlichia*, *Babesia* and *Leishmania* were conducted, with positive results for both *Erlichia* and *Babesia*. It was prescribed to increase the dose of prednisone[4] again [40 m^2/kg], pregabalin[3] [4 mg/kg] was instituted for pain control and doxycycline[7] [8 mg/kg], all via orally.

DISCUSSION

Massive bee attacks can cause serious complications for both human and animals [5,9], due to its' potent venom [19]. Data concerning emergency veterinary care records are scarce. The present work reports a complete and detailed description of a dog's systemic toxic reaction to bee stings, including history, clinical signs, laboratory findings, emergency care and development.

Clinical manifestations following bee attacks can be divided into local reactions, allergic manifestations, anaphylaxis and systemic toxic reactions. The latter is characterized by clinical complications independent from immune reactions, usually after multiple stings [19], as described in the present case.

Edema and erythema were the main signs at the inoculation sites, mostly on the face, tongue and neck. The location is related to dogs' defensive behavior, which also includes ingesting bees [17], as seen in the vomit. The mechanism of edema formation is associated with the hydrolysis of cell membrane phospholipids by phospholipase A_2 (PLA_2), as well as by mast cell degranulation [16] and melittin necrotic activity [3].

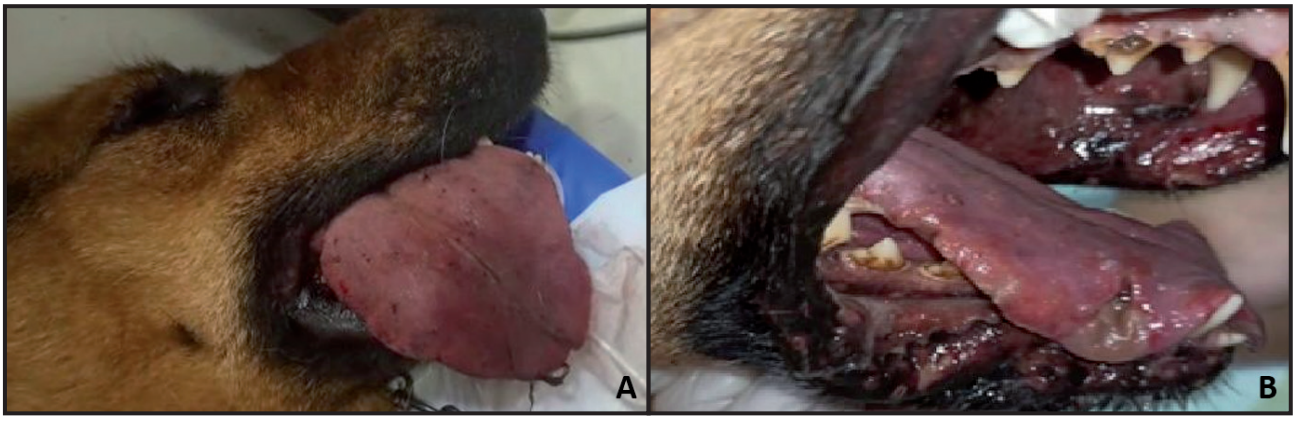

Figure 1. Clinical signs presented in a 6-year-old bitch German Shepperd following massive bee attack. A- Edematous tongue with stings at the 1st day of veterinary care (24 h after the attack). B- Loss of tongue tissue, 7 days after the attack. C- Scarred tongue, 2 weeks after bee sting attack.

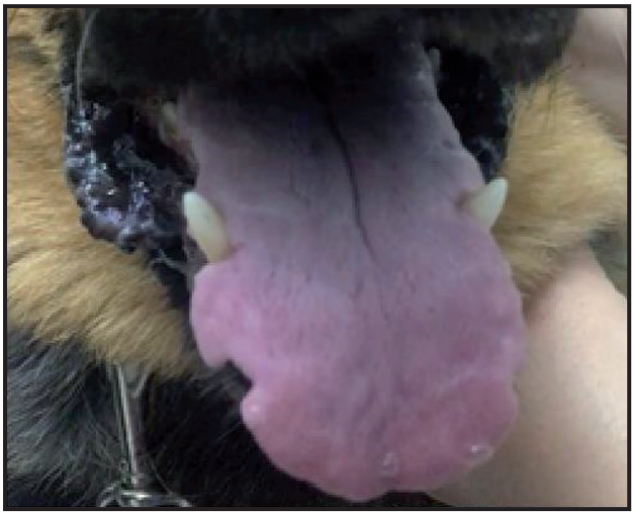

Figure 2. Scarred tongue, 2 weeks after bee sting attack in a 6-year-old bitch German Shepperd.

In order to reduce edema and provide emergency support, oxygen, corticosteroids, analgesic and antihistamines were administered. In addition, adrenaline was also administered to control vasodilation and subsequent edema, promoting a bronchodilator effect and preventing anaphylaxis [14]. Unfortunately, bee antivenom is not yet available in veterinary medicine, however supportive treatment showed significant improvement along the hospitalization period.

During the 9 days hospital stay, several tests were conducted. Blood count showed polycythemia, leukocytosis by neutrophilia and monocytosis, and thrombocytopenia, while biochemical profile showed azotemia. Polycythemia is often associated with hemoconcentration due to dehydration, and was quickly resolved by fluid therapy. Leukocyte count however remained high, despite initiating antibiotic therapy. We believe this immune response is due to the inflammatory potential of the venom and possible complications due to tongue necrosis and diarrhea.

Thrombocytopenia has been described in some other studies [15,16]. There is evidence that this is caused by an immune-mediated reaction, in this case associated not only with poisoning, but with a positive diagnosis for positive results for both *Erlichia* and *Babesia*. The antigenic stimulus and inflammation caused by the venom trigger a type II hypersensitivity reaction [16], leading to immune-mediated thrombocytopenia, in addition to direct vascular damage and consumption by hemorrhage [5,22]. Based on these findings, corticosteroid therapy was performed at immunosuppressive doses.

The most important changes in the case, however, were nephrotoxic. On the 1st day of hospitalization, 24 h after the attack, the patient presented increased values of urea and creatinine. In addition, he presented dark red urine, decreased diuresis during the subsequent days and proteinuria. Thus, we can state that the patient had grade II of acute kidney injury (AKI), according to IRIS [11].

Bee venom causes decreased glomerular filtration rate (GFR) [10] due to toxic-systemic acute tubular necrosis [10], and systemic complications, such as hemolysis and rhabdomyolysis. Renal tubular deposition of components resulting from lysis of erythrocytes and muscle cells leads to ischemia and necrosis. Another contributing factor is systemic hypotension, however, in this case, the patient had no changes in systolic blood pressure.

On the following days of hospitalization, the patient showed significant clinical improvement, and was finally discharged after 9 days, maintaining corticoid therapy, antimicrobial therapy, analgesic and supportive care as recommended [19]. Tongue necrosis was concerning, but progressed well. Two follow up consultations revealed claudication due to polyarthritis. The complication could be a result of the deposition of immunomediated complexes in the joints, due in this case to the bee poisoning [13] and presence of hemoparasites. Therefore, in addition to previous medications, the animal received the antimicrobial agent of choice, doxycycline.

There is a variety of extremely rare and unusual hymenoptera sting circumstances with regard to sting localization, geographic region, massivity of multiple stings, and particularly related to clinical symptoms [20]. These reactions are very infrequent, usually described in isolated case reports. With respect to pathogenesis, the major mechanisms involved are toxic, autoimmune, and other delayed immunological ones. While delayed inflammatory symptoms are considered as delayed hypersensitization or autoimmune entities, generalized rhabdomyolysis and consecutive acute kidney injury is considered a toxic reaction, mostly induced by massive envenomation to wasps or "Africanized" bees.

Immune-mediated nonerosive polyarthritis is believed to be driven by a type III hypersensitivity reaction where immune complexes comprised of antigen bound to antibody accumulate in the joint space. Implicated antigens are typically found in the systemic circulation, but

can originate from within the joint space itself. Systemic immune complexes can arise from a variety of antigenic stimuli, including venoms. In addition, antibodies directed against self-antigens, can also form complexes that accumulate in the joint space. The presence of immune complexes in the joint space activates complement along the synovial membrane and within the synovial fluid. Complement fixation results in tissue damage and the release of cytokines, some of which attract neutrophils. These neutrophils also release cytokines and lysosomal enzymes that cause further tissue damage. In conclusion, although unusual reactions are extremely rare, it is important to keep them in mind [12].

Systemic reactions to bee stings are complex, with minor to extensive potential lesions and complications. The present report describes full emergency and clinical care following massive attack in a dog, highlighting to possible complications tongue necrosis and arthritis, reinforcing the importance of follow-up consultations.

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