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Chronic lead intoxication in a jaguar (*Panthera onca*) shot with round lead pellets – case report

[*Intoxicação crônica por chumbo em uma onça pintada (*Panthera onca*) alvejada com projéteis de chumbo – relato de caso*]

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ABSTRACT

Lead is a heavy metal and an important cause of acute or chronic toxicosis in humans, domestic, and wild animals. This report aims to describe a case of chronic lead poisoning in a jaguar (*Panthera onca*) kept under human care that was rescued from the wild environment. The animal was rescued in poor condition in 2004 and kept under human care at the Belo Horizonte Zoological Garden (Minas Gerais, Brazil) until 2020, when it presented with anorexia, vomiting and ataxia. Over the past years the animal had episodes of anemia and increased serum urea and creatinine. Radiography demonstrated 21 radiopaque projectiles on the right side of the face. At necropsy there were multiple projectiles surrounded by fibrous tissue in the subcutaneous of the right side of the face, fibrinous peritonitis, multiple gastric ulcers, and melena. The lead dosage was performed using the atomic absorption spectrometry technique using renal tissue collected at necropsy, with a result of 908 ppb (µg/kg). The findings of projectiles associated with the dosage of lead above the reference limits allow the diagnosis of chronic intoxication in this case.
Keywords: heavy metals, anemia, toxicosis, hunting

RESUMO

*Chumbo é um metal pesado e uma causa importante de toxicose crônica no homem e em animais domésticos e selvagens. Este relato teve como objetivo descrever um caso de intoxicação crônica por chumbo em uma onça-pintada (*Panthera onca*), mantida sob cuidados humanos, que foi resgatada de seu ambiente natural em 2004, em pobre condição corporal. O animal foi encaminhado ao zoológico de Belo Horizonte (MG, Brasil), onde permaneceu até 2020, quando desenvolveu anorexia, vômitos e ataxia. Durante os últimos anos, o animal teve episódios de anemia e aumento da concentração sérica de ureia e creatinina. O exame radiográfico demonstrou 21 projéteis radiodensos na face direita. À necropsia, foram observados vários projéteis envoltos por tecido conjuntivo no subcutâneo da face esquerda, peritonite fibrinosa, múltiplas úlceras gástricas e melena. A concentração de chumbo foi determinada por espectrometria de absorção atômica em amostras de tecido renal, indicando 908 ppb (µg/kg). O achado de projéteis de chumbo associado à elevada concentração de chumbo é compatível com intoxicação crônica por chumbo neste caso.*

Palavras-chave: metais pesados, anemia, toxicose, caça

INTRODUCTION

Lead is a heavy metal that is highly toxic to humans and animals, and acute or chronic poisoning can occur. Because it is not biodegradable, lead persists in the environment and ends up causing the process of biomagnification, which predisposes to intoxication of top predators (Mitra *et al*., 2017). Poisoning in wild animals occurs predominantly by ballistic accident in cases of hunting or

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ingestion of projectiles in animal carcasses (LaDouceur *et al*., 2015). Ingestion of fishing weights and environmental contamination due to mining also represent important sources of intoxication (Francisco *et al*., 2003).

When lead enters the body, it can cause leademia for approximately 40 days, and after that, if the primary source is removed, the lead blood levels tend to decrease. With chronicity, there is metal deposition in the soft tissues, mainly in the liver and kidneys, which are considered essential tissues for the diagnosis in necropsy cases. Importantly, lead also accumulates in bones, which are suitable for diagnostic purposes in cases with advanced putrefaction (Mitra *et al*., 2017).

The most important clinical signs associated with acute lead toxicosis are neurological changes including ataxia and nystagmus; gastrointestinal changes including vomiting, diarrhea, and melena; acute renal failure; anemia; among others. Clinical signs associated with chronic lead toxicity may be absent, but when present they may include anemia, renal failure, immunosuppression, cardiac changes, gastrointestinal changes including persistent vomiting due to gastroesophageal ulcers, and central nervous system involvement with lethargy, ataxia, and seizures (Høgåsen *et al*, 2016; Knight and Kumar, 2003).

The jaguar (*Panthera onca*) is the third largest feline in the world. It occurs in several regions of the Americas. In Brazil, it occurs predominantly in the Amazon region, Pantanal, Cerrado, and Atlantic Forest. In a general context the jaguar is considered Near Threatened by the IUCN Redlist, and the main threats are hunting and habitat loss (Quigley *et al*., 2017). The aim of this report was to describe a case of chronic lead poisoning in a jaguar kept under human care that was rescued from the wild with multiple subcutaneous lead projectiles.

CASE REPORT

A juvenile male jaguar was found debilitated in Governador Valadares, State of Minas Gerais (Brazil) in 2004, sent to a local zoo in the city of Ipatinga, and later admitted to the Belo Horizonte Zoological Garden (BH Zoo, Belo Horizonte, Minas Gerais, Brazil), where it was kept under human care with two other males of the same species. The animal was then introduced into the BH Zoo breeding program, but never bred successfully. In July 2020, the jaguar presented with anorexia, motor incoordination, nystagmus, and vomiting. The animal was anesthetized for clinical evaluation and imaging tests. Radiographic examination revealed 21 radiopaque projectiles with approximately 0.5 cm in diameter in the right side of the face, predominantly affecting the maxillary and zygomatic arch (Fig. 1). It was reported that over the years the animal had episodes of anemia with decreased hematocrit, hemoglobin, and red blood cell counts (Fig. 2) (Adania *et al*., 2014). There were also sporadic episodes of increased serum urea and creatinine (Fig. 3) (Adania *et al*., 2014). Three days after the discovery of the projectiles, blood and urine were collected for lead measurement by atomic absorption spectrometry technique, but both results were below the detection limit. The animal was hospitalized for follow-up and clinical evaluation, but due to poor prognosis and evolution was euthanized.

A conservative necropsy was performed to preserve the animal for taxidermy, which prevented the macroscopic and histopathological evaluation of the skeleton and bone marrow. Grossly, visible mucosae were moderately pale and there was an ulcer on the ventral surface of the tongue. The jaw and right zygomatic arch had projectiles of approximately 0.5 cm encapsulated by fibrous tissue (Fig. 4A). In the abdominal cavity there was 500 mL of cloudy, yellowish fibrinous exudate. The visceral and parietal peritoneum were intensely hyperemic with fibrin deposition on the serosa of abdominal organs. The stomach mucosa was slightly swollen and there were multiple depressed areas of ulcerations (Fig. 4B). Melena was observed in the small and large intestines. Tissue samples were collected and fixed in 10% buffered formalin for 48 hours, embedded in paraffin, sectioned in a microtome (3 µm-thick sections), and stained with hematoxylin and eosin or Gram stain.

Figure 1. Jaguar (*Panthera onca*). Radiograph, ventro-dorsal and latero-lateral view. Right maxillary and zygomatic arch region with 21 radiopaque projectiles of approximately 0.5 cm in diameter.

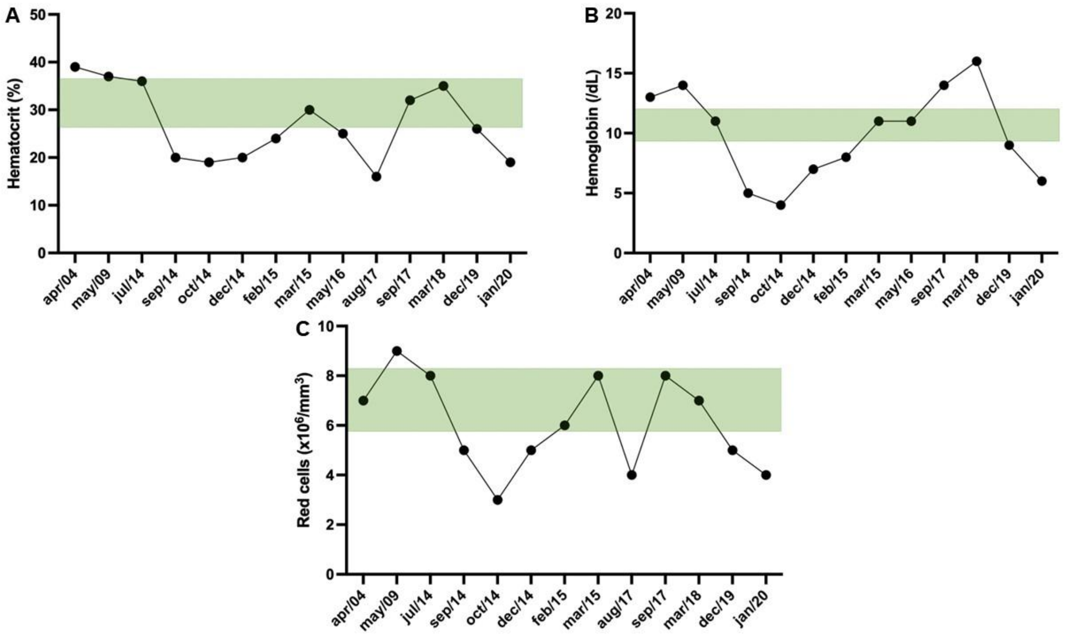

Figure 2. A) Hematocrit (%) of individual samples collected between April 2004 and January 2020. The green band represents the reference values. B) Hemoglobin values (g/dL) of individual samples collected between April 2004 and January 2020. The green band represents the reference values. C) Red cell count ($x10^6$/mm3) of individual samples collected at times between April 2004 and January 2020. Green band represents the reference values. Reference values according to Adania *et al.*, 2014.

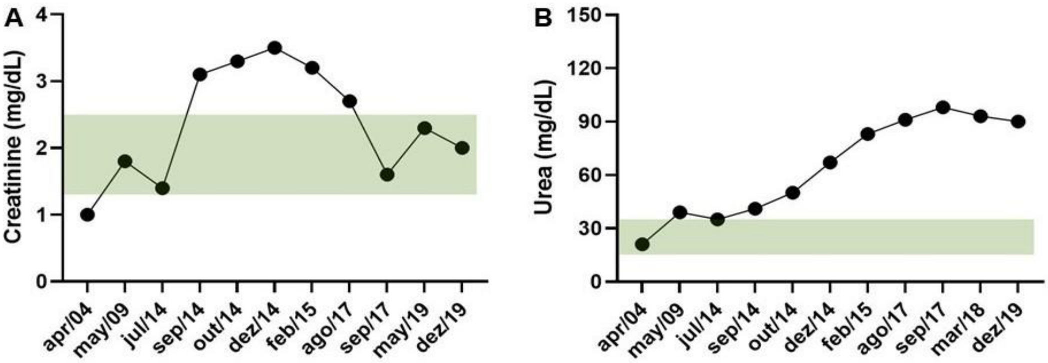

Figure 3. A) Individual serum creatinine values (mg/dL) between April 2004 and December 2019. Green band represents the reference values. B) Individual serum urea values (mg/dL) between April 2004 and December 2019. Green band represents the reference values. Reference values according to Adania *et al*., 2014.

Histologically, the gastric mucosa had multifocal areas of ulceration, necrosis, and accumulation of neutrophils, with intralesional bacteria (ulcerative gastritis). In the submucosa, adjacent to the ulcers, blood vessels were occluded by organized fibrillar eosinophilic material, characterizing thrombosis. Adjacent mesentery had focally extensive thickening associated with marked inflammatory infiltrate composed of macrophages, lymphocytes, neutrophils, and plasma cells. There was also hyperplasia of mesothelial cells and multifocal areas of fibrin deposition interspersed with neutrophils, macrophages, and lymphocytes with many Gram-positive cocci (bacterial fibrinous peritonitis). The visceral peritoneum on the liver, spleen, and intestines had moderate multifocal neutrophilic infiltrate with fibrin deposition and sometimes aggregates of Gram-positive cocci. In the kidneys, glomeruli had diffused and marked thickening of basal membranes in Bowman's capsule (Fig. 4C), and sometimes also affecting the mesangium (membranous glomerulopathy). Testicles had seminiferous tubules with marked decreased cellularity and accumulation of weakly homogeneous eosinophilic material (Fig. 4D), interstitium with multifocal accumulation of adipocytes and testicular meatus with proliferation of fibrous connective tissue between the efferent ducts (testicular hypotrophy).

During necropsy, fragments of kidneys were sampled and sent for lead measurement by the atomic absorption spectrometry technique, which indicated a value of 908 ppb (µg/kg) of lead in renal tissues. The peritoneal fluid was sent to the bacteriology laboratory with bacterial isolation compatible with a mixed population of *Staphylococcus lentus*, *Escherichia coli*, and *Aeromonas* sp. Samples were also sent to the virus laboratory for investigation of Feline Infectious Peritonitis Virus (FIP) by reverse transcription followed by polymerase chain reaction (RT-PCR), with negative results.

DISCUSSION

There are currently no reference values for lead levels in tissues of wild felids. In this case, limits of residues recommended by the Brazilian Ministry of Agriculture (MAPA) were used as a reference (Brasil, 2018). This normative instruction establishes the value of 500 ppb (µg/kg) of tissue as the maximum value allowed in renal tissues of some mammals, such as swine, cattle, and goats. The finding of projectiles encapsulated by fibrous connective tissue associated with the detection of elevated levels of lead in the kidneys (908 ppb) supported the diagnosis of chronic lead intoxication in this case. Reports of lead poisoning in wild felids are scarce, with two previously reported cases. The first report was a case of poisoning of a free-ranging puma (*Puma concolor*) by ingestion of lead bullets from a carcass of a prey (Burco *et al*., 2012) and the second was a case of poisoning of two captive cheetahs (*Acinonyx jubatus jubatus*) by ingestion of game meat with lead projectiles (North *et al*., 2015).

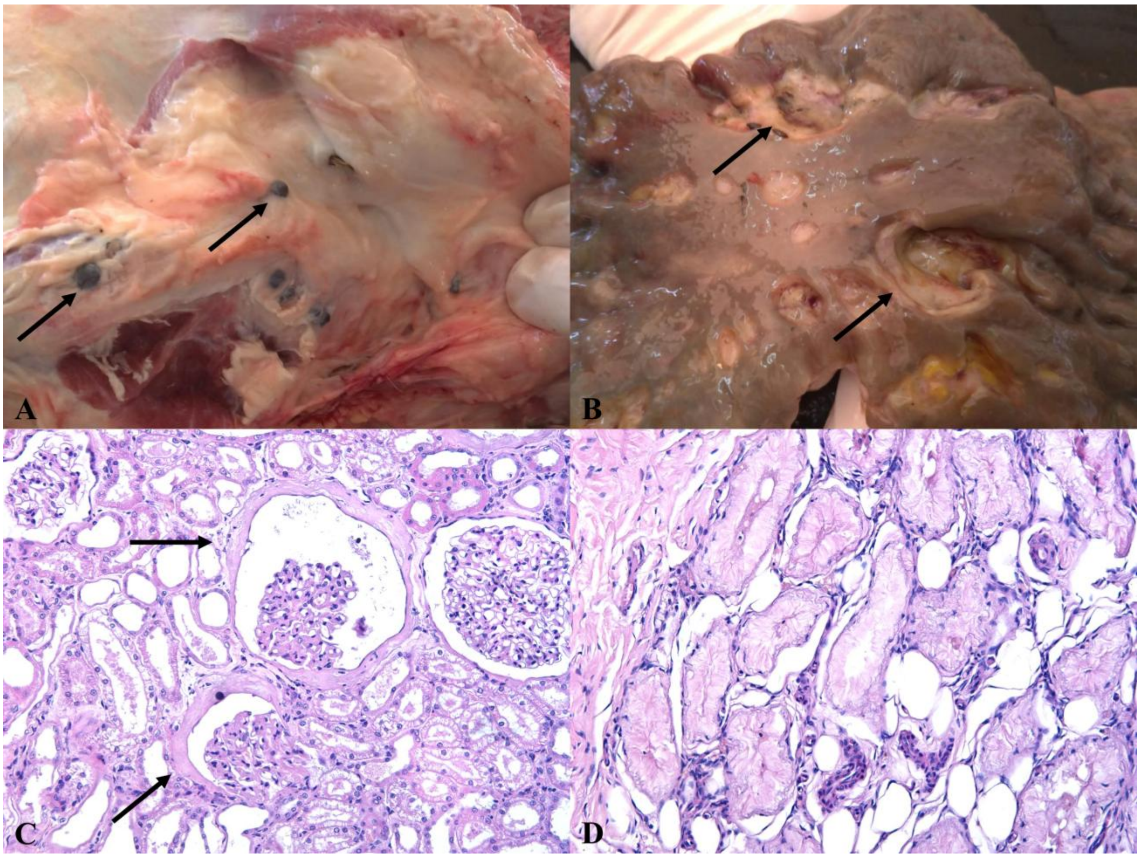

Figure 4. Jaguar (*Panthera onca*) (A-D). A) Subcutaneous of the right zygomatic arch and maxillary region, arrows show multiple spherical, black, hard projectiles measuring approximately 0.5 cm, partially encapsulated by fibrous connective tissue. B) Stomach, arrows show multiple ulcerations with irregular edges in the mucosa (ulcerative gastritis). C) Kidneys, arrows indicating thickening of the basal membrane of the Bowman's capsule by deposition of dense eosinophilic material (membranous glomerulopathy), HE, 200x magnification. D) Testis, seminiferous tubules with absence of cellularity and accumulation of poorly homogeneous eosinophilic material (testicular hypotrophy), HE, 200x magnification.

As large felines are top predators, they are predisposed to lead poisoning due to biomagnification or direct consumption of bullets (Millán *et al*., 2008). As in the present case, poisoning by ballistic accidents resulting from hunting is widely reported in birds and other wild mammals (LaDouceur *et al*., 2015). The detection of high levels of lead in the blood is considered the gold standard for the diagnosis of poisoning in live animals, but in chronic cases lead blood levels tend to decrease due to bioaccumulation of lead in soft tissues and bones (Knight and Kumar, 2003), which explains the concentration of lead in the blood and urinary tract are within the normal range in this case.

Recurrent anemia in this animal may be considered a manifestation of plumbism, since this element is known to be responsible for impairing hematopoiesis by inhibiting heme production enzymes and reducing the half-life of the erythrocyte by increasing the reactive oxygen species (ROS) and inhibition of endogenous antioxidant activity (Mitra *et al*., 2017). Human studies have shown that lead leads to infertility with low spermatogenesis due to increased ROS. In this case, the animal had a history of infertility, which was associated with testicular hypotrophy that may also be attributable to chronic lead poisoning, but larger studies should be carried out for a better understanding of the effect of this metal on the reproductive system of wild felids (Mitra *et al*., 2017).

Despite the absence of current renal biochemistry tests, laboratory findings of sporadic increases in urea and creatinine associated with ulcerative glossitis, ulcerative gastritis with thrombosis, and membranous glomerulopathy support the diagnosis of chronic kidney disease. Studies have shown that in cases of acute intoxication, proximal tubular damage predominates, and animals or humans can develop Fanconi's syndrome. In chronic intoxication, glomerular damage, as seen in this case, is the most frequent, as well as interstitial lesions of nephritis and fibrosis (Flora *et al*., 2012; Mitra *et al*., 2017). Lead has action on the gastrointestinal system, leading to vomiting, severe abdominal pain, and gastroesophageal ulcers. In our case, lead poisoning associated with renal lesions may have contributed to the formation of gastric ulcers (Høgåsen *et al*, 2016).

Although we did not visualize any areas of perforation, bacterial peritonitis can be associated with a perforated gastric ulcer, since in many cases perforation may not be recognized grossly (Uzal *et al*., 2016). Peritonitis caused by the feline coronavirus was ruled out based on RT-PCR analysis. No lesions were observed in the central nervous system, and the clinical signs presented may be due to peritonitis and ulcerative gastritis.

CONCLUSION

Ballistic accidents represent an important cause of lead poisoning in felids and other wild animals. Chronic intoxication may be silent, and clinical signs may be mild or absent. Lead poisoning should be among the differentials in wild felids that present with recurrent anemia. The absence of high levels of lead in the bloodstream does not rule out chronic intoxication, and the proper collection of samples at the time of necropsy is essential for a correct diagnosis. The establishment of reference parameters for the dosage of lead in wild animals is necessary for a better understanding of intoxication in these species.

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