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Caption: Stripe-backed Weasel *Mustela strigidorsa*. Medium—digital, Software—procreate, Device—iPad + Apple pencil © Dhanush Shetty.

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SHORT COMMUNICATION

Is release of rehabilitated wildlife with embedded lead ammunition advisable? Plumbism in a Jaguar *Panthera Onca* (Mammalia: Carnivora: Felidae), survivor of gunshot wounds

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Abstract: Lead poisoning is a threat to wildlife, particularly after ingestion of lead ammunition derived from hunting activities. Little information, however, is available concerning plumbism in wild animals that survive the trauma associated with gunshot wounds. This study presents a possible example of lead intoxication by embedded pellets in a Jaguar *Panthera onca* nineteen months after being injured by a shotgun blast. In addition, the possible path of incorporation of lead into the trophic chain after the eventual release and death of an impacted animal, thereby expanding and prolonging the toxic effects of lead ammunition, is discussed. Direct intoxication by ammunition retained in the body of wild animals, as well as the indirect impacts on predators and scavengers that consume their flesh, should be sufficient reasons to reconsider the release of individuals with embedded lead ammunition into the wild.

Keywords: Blood lead levels, endangered species, lead-free ammunition, trophic web.

The Jaguar *Panthera onca,* is the largest American felid, and the only living representative of the genus *Panthera* in the New World (Caragiulo et al. 2016). Historically distributed from the southwestern United States to southern Argentina, Jaguars inhabit a wide range of ecological zones, from tropical moist forests, to xeric shrublands, to tropical dry forests, to grasslands and savannas (Sanderson et al. 2002). The IUCN Red List classifies the species globally as Near Threatened since 2002; the population trend is decreasing due to habitat loss and direct human persecution (Quigley et al. 2017). Currently, although the species is included in Appendix I of the Convention on International Trade in Endangered Species (CITES 2019), threats have continued or intensified at local and regional scales, and Jaguars have already disappeared from 55% of their historical range. The majority of subpopulations are Endangered

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or Critically Endangered (De la Torre et al. 2018). In Ecuador, where hunting remains one of the main threats to the species, two subspecies are separated by the Andean highlands, the Endangered *Panthera onca onca* from Amazon rainforest (Espinosa et al. 2011a), and the Critically Endangered *P. onca centralis* from the western coast (Espinosa et al. 2011b).

Lead ammunition is highly toxic for wildlife, especially in long-lived scavengers and predators (Gil-Sánchez et al. 2018). Intoxication of wildlife following ingestion of lead ammunition has long been recognized (Pain et al. 2019), contributing to population decline of some threatened species (Fernandez et al. 2011; Finkelstein et al. 2012; Garbett et al. 2018). Nonetheless, little is known about the impact of retained lead projectiles from gunshot wounds, despite a high incidence of embedded lead in wild animals secondary to hunting injuries (LaDoucer et al. 2015, Berny et al. 2017). This study describes lead poisoning through retained ammunition in an Endangered Amazon Jaguar subsequent to its recovery from multiple firearm injuries, and the possible incorporation of lead into the trophic chain after incidental ingestion by scavengers.

Material and Methods

On 31 October 2016, a juvenile female Jaguar was transferred from the province of Sucumbíos in northeastern Ecuador to the Wildlife Hospital TUERI of Universidad San Francisco de Quito (USFQ) for evaluation of injuries caused by a shotgun blast. Radiographic series (Sharp Ray LWX-20P) revealed the presence of 18 pellets scattered dorso-anteriorly; six of these were removed surgically (Image 1). The patient gradually improved and was transferred to the recovery center to complete the rehabilitation process two months after admission. In the following nine months, the Jaguar exhibited natural behaviors in terms of hunting and fishing, as well as aversion to and flight from human presence, which indicated excellent potential for successful release into the wild. Eleven months after her original injuries, the Jaguar was captured from its enclosure for clinical evaluation and to assess the possible reabsorption of embedded lead; blood lead levels (BLL) were measured by atomic absorption spectrophotometry (Buck Scientific 210VGP).

Showing no indications of complicating factors, the patient was clinically discharged but still held in

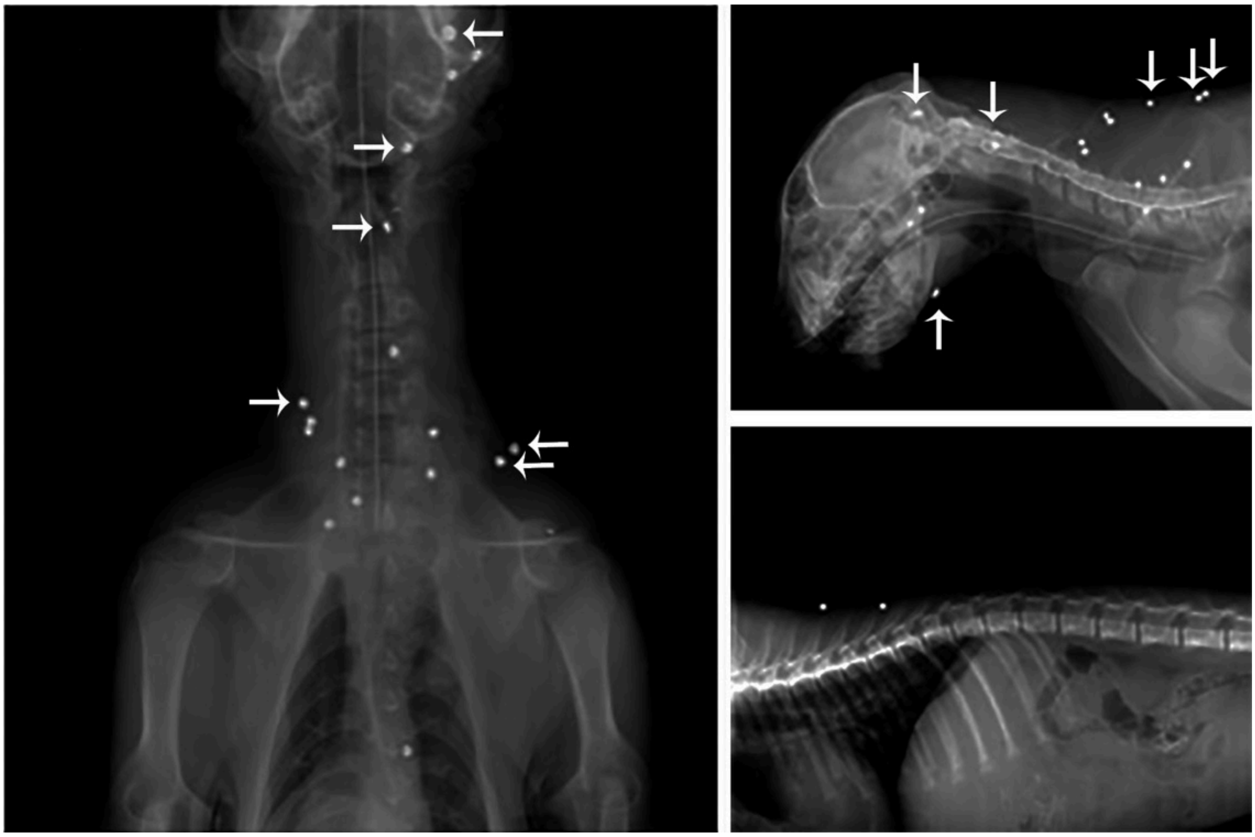

Image 1. Radiographs showing the presence of 18 lead pellets scattered dorso-anteriorly in a juvenile female Jaguar. Arrows indicate the six pellets removed surgically.

natural-setting captivity while her eventual release into a remote area of Yasuní Biosphere Reserve was planned. In the following eight months, she was captured twice more, specifically for the placement of a satellite collar (Telonics TGW-4577-4) for post-release monitoring, and ultimately to evaluate her overall condition for transfer to the release site. Following release on 21 May 2018, the Jaguar was tracked for four days after which the satellite device stopped transmitting movements; the animal was found dead four days later. The carcass presented an advanced state of decomposition and was being scavenged by vultures at the time of encounter. Remains were transferred to Wildlife Hospital TUERI for forensic analysis, including radiology (Sharp Ray LWX-20P) and scanning electron microscopy (JEOL JSM-IT300LA) of bones. Blood samples collected on the day of release were analyzed post-mortem by atomic absorption spectrophotometry (Buck Scientific 210VGP) to determine the possible reabsorption of embedded lead into general circulation.

Results

As previously mentioned, after surgery, twelve lead pellets remained embedded in the Jaguar's body; none within the digestive system or skeletal articulations (Image 1), sites classically considered to present elevated risks (Eward et al. 2011). Eleven months later, no clinical symptoms of lead intoxication were identified, and BLL measurement was negative to the sensitivity limit 0.001 µg/dl. After release, satellite tracking showed movement for four days – 2.7, 1.5, 1.2, and 0.3 km/day, respectively. The advanced state of decomposition presented by the carcass when located only allowed radiological examination; no superficial evidence of the cause of death could be ascertained due to the putrefied condition of the body. None of the 12 pellets remaining in the animal's body were detected by radiographic series. Scanning electron microscopy showed no traces of lead in the bone samples. Atomic absorption spectrophotometry analysis of blood samples collected on the day of the release revealed high BLL (1,223 µg/dl) in noteworthy contrast to earlier samples.

Discussion

Embedded lead has been identified as a risk factor causing plumbism in humans and experimental animals, but it has been poorly investigated in wildlife (LaDoucer et al. 2015; Berny et al. 2017). According to our knowledge, there are no data that relate the presence of embedded projectiles with BLL in wild mammals, but in humans, values greater than 25-40 µg/dL can cause symptoms that range from quite mild to coma and death (Bustamante & Macias-Konstantopoulos 2016). In the present study, no obvious outward symptoms were identified during the time that the Jaguar remained in captivity. Nevertheless, although BLL were not detected during the first eleven months, eight months later they reached one of the highest values recorded to date for a wild felid (Burco et al. 2012; North et al. 2015). This could be due to the type of exposure. In the case of chronic exposure, symptoms appear progressively and become incrementally more severe as time passes. Conversely, severe symptoms can erupt suddenly in acute exposures (Kim et al. 2015). Therefore, absence of evident clinical symptoms, and sudden death of the Jaguar, may have been due to acute absorption of lead.

Pain, weakened muscle strength, sensory abnormalities and brain inflammation can appear as acute symptoms in plumbism. More severe manifestations occur at very concentrated exposures, and symptoms abruptly worsen to include delirium, loss of muscular coordination, convulsions, ataxia, paralysis, coma and death (Sanders et al. 2009; Flora et al. 2012). Following release, satellite tracking revealed that the distance moved by the animal progressively decreased in the subsequent days (2.7, 1.5, 1.2, and 0.3 km/day).

In an effort to corroborate the hypothesis of acute exposure, a bone analysis was performed seeking to determine the presence of lead. The accumulation of lead in bones is indicative of long-term exposure due to its extended residence time, in contrast to BLL that is used to measure recent exposure because of the short half-life of lead in the blood (Green & Pain 2019). Scanning electron microscopy did not detect lead residues in bone samples, confirming that the embedded reabsorption would not have been chronic.

In most cases of plumbism, lead is ingested and absorbed into the bloodstream through the intestinal tract. Acute onset of nervous symptoms is a potential condition of captive felids fed hunted game animals (North et al. 2015). In our case, the Jaguar did not receive hunted meat or any other type of food that could contain traces of lead, making it is impossible that the poisoning occurred in this way; any BLL should come through reabsorption from the ammunition embedded in its body. In human cases, lead toxicity with intra-articular retained ammunition is indeed considered a risk, but extra-articular embedded lead, when difficult to extract, is routinely permitted to remain in tissues indefinitely without surveillance for lead toxicity. However, lead toxicity associated with extra-articular retained ammunition, although uncommon, may be asymptomatic and difficult to diagnose yet debilitating and potentially lethal (Eward et al. 2011; Grasso et al. 2017). This fact could corroborate our findings in the present case. Although there seems to be no clear relationship between the amount of lead retained, residence time or location in the human body, embedded lead cannot be considered inert or safe (De Araújo et al. 2015).

Primary treatment in cases of retained lead ammunition usually includes chelation, followed by complete surgical removal of retained projectiles, in order to prevent systemic toxicity (Bustamante & Macias-Konstantopoulos 2016). Nevertheless, sometimes the elimination of projectiles would require complicated surgical approach resulting in extensive tissue dissection and high morbidity in a patient weakened by trauma (De Araújo et al. 2015). When surgical removal of ammunition fragments is contra-indicated, there are currently no long-term treatment methods available, since the source of exposure remains in the body, and prolonged chelation would cause adverse health effects such as hepatotoxicity or nephrotoxicity (McQuirter et al. 2004; Flora & Pachauri 2010). Therefore, these patients should be considered at chronic risk for lead poisoning and monitored periodically (Moazeni et al. 2014).

Finally, in the radiological tests performed on the Jaguar carcass, none of the 12 embedded pellets was detected. A likely explanation is incidental ingestion by scavengers; three species of vultures were identified next to the Jaguar cadaver: the American Black Vulture *Coragyps atratus*, the Greater Yellow-headed Vulture *Cathartes melambrotus*, and the King Vulture *Sarcoramphus papa*. Therefore, lead projectiles retained in the Jaguar's body could have had devastating effects not only for the Jaguar itself, but also for other species upon entering the trophic web through carrion consumers. In South America, with lead poisoning being a major widespread conservation threat for the Andean Condor *Vultur gryphus* (and probably for other sympatric carnivores also), urgent conservation actions to reduce this toxin in the wild are necessary (Wiemeyer et al. 2017). In this sense, evidence on the adverse effects of the use of lead ammunition on wildlife is ample. Because the change to non-toxic alternatives is possible and would allow important benefits for nature conservation (Kanstrup et al. 2018; Cromie et al. 2019), the strategy should be considered and implemented on a much broader scale.

In conclusion, some limitations of this work have been the lack of more thorough BLL monitoring during the time that the patient remained in captivity due to the difficult handling of the species, and the impossibility of assessing the presence of lead in other soft tissues after death because of the advanced state of decomposition in which the carcass was found. According to the authors' knowledge, the present study suggests for the first time that the presence of embedded pellets can be associated with a significant blood lead concentration in a wild felid. This situation reinforces the need for better understanding of the toxic effects of lead in wildlife surviving gunshot wounds. Until then, each patient with lead ammunition retained in its body should be considered at chronic risk for itself and a potential danger to other species that may eventually feed on it. Therefore, release of individuals with embedded lead projectiles should be carefully considered by wildlife managers when complete removal of lead fragments is not possible.

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