

Long-term lead intoxication of Griffon Vulture (*Gyps fulvus* Hablizl, 1783) supposedly the result of illegal shooting

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Abstract The European Griffon Vulture *Gyps fulvus* is a large-sized scavenger exploiting carcasses of livestock and wild ungulates and thus having a paramount importance in the natural ecosystems. In this study, we report on an adult Griffon Vulture detected with lead levels in the bones over the threshold. After two years of tracking, the bird died. The corpse's clinical examination and radiography detected the presence of two embedded lead pellets from a healed gunshot wound in its right wing. Quantitative laboratory analysis of lead in bone and liver samples evidencing subclinical/chronic lead intoxication of the Griffon Vulture could potentially be a result of the long-term exposure to the lead originating from the pellets in its wing.

Keywords: scavenger, toxicity, heavy metal, Eastern Rhodopes, embedded lead pellets

Összefoglalás A fakó keselyű (*Gyps fulvus*) egy nagytestű, dögevő ragadozómadár-faj, amely haszon- és vadállatok elhullott tetemein táplálkozik és ezáltal fontos szerepet tölt be a természetes ökoszisztémában. Ebben a tanulmányban egy olyan öreg példány elhullásáról számolunk be, amelynek véréből már egy korábbi mintavétel során is határértéken felüli ólomkoncentráció mutatkozott. A műholdas jeladóval ellátott egyed két évvel később elpusztulva került meg. A tetem boncolását és röntgenvizsgálatát követően bebizonyosodott, hogy a madár jobb szárnyában két, korábbi lövésből származó ólomsörét volt beágyazódva. A laborvizsgálatok során a fakó keselyű csontszövetéből és májából származó minták egyaránt szubklinikai/krónikus ólommérgezésre utaló koncentrációt mutattak, amelyet feltehetően a madár szárnyában hosszú ideig jelenlévő sörétszemek okoztak.

Kulcsszavak: dögevők, toxicitás, nehézfémek, Kelet-Rodope, beágyazódott ólomsörét

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Introduction

The Eurasian Griffon Vulture *Gyps fulvus* is a large Old World vulture that scavenges carcasses of livestock and wild ungulates (DeVault *et al.* 2003). Its breeding distribution extends from Kazakhstan and Nepal in the east, throughout the Caucasus, southern Europe and the Iberian Peninsula to the west (BirdLife International 2020). Adult Griffon Vultures

are mostly resident, while juveniles are primarily nomadic, some exhibiting migratory behaviour, overwintering in Africa and the Middle East (del Hoyo *et al.* 1994, McGrady & Gavashelishvili 2006, Arkumarev *et al.* 2019). On the Balkan Peninsula, the species breeds in Bulgaria, Greece, North Macedonia, Serbia and Croatia (Sušić 2004, Xirouchakis & Tsiakiris 2009, Grubač 2013, Sušić & Radek 2013, Veleviski *et al.* 2013, Demerdzhiev *et al.* 2014). The Griffon Vulture population in Bulgaria has steadily increased in the past 30 years as a result of intensive conservation actions and reintroduction programmes (Dobrev & Stoychev 2013, Demerdzhiev *et al.* 2014, Stoynov *et al.* 2018).

Lead is a highly toxic heavy metal with an important impact on bird populations. Absorption of a low concentration of lead may result in a wide range of sublethal effects in animals, and higher concentrations may result in acute intoxication and consequent death. It is an accumulative metabolic poison that is non-specific, affecting a wide range of physiological and biochemical systems including the hematopoietic, vascular, nervous, renal, immune and reproductive systems (Franson & Pain 2011, Pain *et al.* 2019).

Lead poisoning originating from ammunitions is a well-known threat for wildlife species on a global scale (Plaza *et al.* 2018, Pain *et al.* 2019). Evidence of lead intoxication negatively affecting raptor species are documented for many endangered birds such as the Californian Condor *Gymnogyps californianus*, the Cinereous Vulture *Aegyptius monachus*, the Egyptian Vulture *Neophron percnopterus* on the Balkans and the Bearded Vulture *Gypaetus barbatus* in North America, Spain and the Alps (Fry 2003, Rodriguez-Ramos *et al.* 2008, Bounas *et al.* 2016, Ganz *et al.* 2018).

Several mechanisms of lead intoxication are reported in wild birds. Lead from ammunitions dispersed in the environment can be swallowed (Beintema 2001, Scheuhammer *et al.* 2003), and/or embedded within the bird's tissues after shooting and consequently, accumulated in the blood over time (Finkelstein *et al.* 2014, LaDouceur *et al.* 2015).

Griffon Vultures feed on various game species found in the landscape, which puts them at high risk of chronic or acute lead poisoning by ingesting lead pellets embedded in tissues of the shot animals (Carneiro *et al.* 2016, Arrondo *et al.* 2020). Juvenile and immature vultures spend their first years of life wandering vast areas in the Middle East, Africa and Asia (Arkumarev *et al.* 2019) and can become victims of illegal shooting. Some wounded birds survive and continue their way south carrying lead pellets embedded in their tissues. Illegal killing is among the main threats and of great conservation concern in the Eastern Mediterranean countries where annually thousands of raptors and other migratory birds are shot on their way to Africa (Brochet *et al.* 2019). Demerdzhiev *et al.* (2014) reports 15.79% of the Griffon Vultures found dead in Bulgaria from 1979 to 2011 as victims of illegal shooting. However, this threat is considered to bear a low impact on the Griffon Vulture globally (Botha *et al.* 2017), but yet, there are numerous cases of killed or injured vultures.

The lead intoxication of Griffon Vulture in various aspects is well studied in Spain (García-Fernández *et al.* 2005, Espín *et al.* 2014, González *et al.* 2017, Arrondo *et al.* 2020). However, the impact and evidence of this threat have not been reported from the Balkans. We present here a case of long-term chronic lead intoxication of a wild Griffon Vulture, tracked with a GPS transmitter and found dead in the Eastern Rhodopes, Bulgaria.

Materials and methods

The Griffon Vulture was captured on 25.05.2017 via a walk-in trap in the Eastern Rhodopes, Bulgaria. Based on its plumage, the vulture was aged as an adult (Forsman 2003). Based on a blood DNA sample, the vulture was sexed as a female. The bird was measured at the time of trapping obtaining weight and morphometrics. The vulture was marked with a colour wing tag, a standard metal ring, and a colour ring, to ease its identification in the wild. Blood and saliva samples were collected for toxicological and microbiological analysis. The Griffon Vulture was also fitted with a solar-powered 57g GSM/GPS and accelerometry transmitter (E-Obs GmbH; 132 Munich, Germany) attached as a backpack configuration with 11.2 mm Teflon ribbon (Arkumarev 2020).

The vulture died on 17.04.2019 in the Eastern Rhodopes, Bulgaria. The body was recovered on the next day in good overall condition. The bird was transported for admission to the Wildlife Rehabilitation and Breeding Centre “Green Balkans” – Stara Zagora (WRBC), where initial external examination and body weight measurement were conducted. A consequent radiography was assigned. A standard gross pathological examination followed. The necropsy was executed to investigate the reasons that led to its death. Sample from femur bone and liver to test for lead (Pb) contents were obtained additionally and sent for quantitative lead tests to an accredited laboratory in Bulgaria.

Results

The Griffon Vulture was tracked for 692 days in 2017–2019. This period covers partially or entirely three breeding seasons (2017, 2018, 2019). During this period, no successful breeding was recorded by either telemetry data or by visual observations.

The concentration of Pb in the blood of the bird taken during the tagging was 384.85 ng/g (0.385 mg/kg) dry weight (dw). The vulture weighed 7.6 kg. At the time of admission at WRBC, the corpse of the bird weighed 5.880 kg. During the gross external examination, no clues for electrocution, collision, acute poisoning or poaching were recognized.

The radiography detected two foreign metal particles in the bone of the right wing, supposed to be lead pellets (Figure 1). No specific gross organ

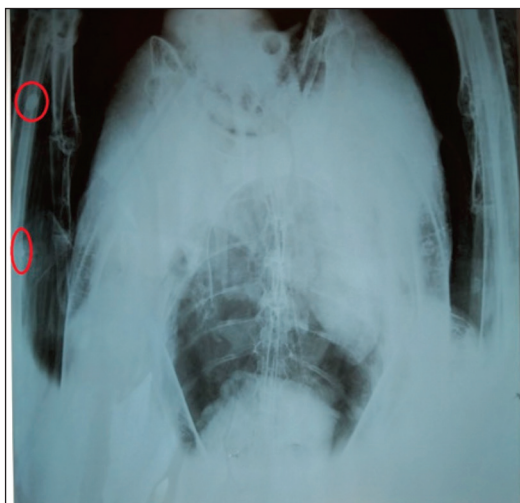


Figure 1. Ventrodorsal X-ray image of the Griffon Vulture with two lead pellets in the right wing

1. ábra A fakó keselyű ventrodorzális nézeti röntgenfelvételén jól kivehető a jobb szárnyban lévő két sörétszem

lesions were detected during necropsy, however, evidence for hepatic necrosis was observed. Visual inspection of the reproductive organs showed atrophy. Laboratory findings indicated the presence of lead in the analyzed femur and liver samples. The post mortem bone sample resulted in 11.58 ± 1.16 mg/kg dw of Pb in the tissue. Lead levels in the liver tissue were 4.06 ± 0.41 mg/kg dw.

Discussion

In this paper, we document a case of a Griffon Vulture from the Balkan population that was a victim of an illegal shooting and carried lead pellets inside its body. Usually, a period of at least two months is necessary for calcification of bones after injury (Paskalev 2013). The bird was tracked and closely monitored with a GPS transmitter for two years. During this period, abnormal behaviour was not observed, which might suggest that the vulture was shot or injured. This indicates that the pellets have been embedded in the bird's right ulna for a long time. We suggest that the bird was already having the lead pellets calcified when it was captured and tagged as it had the highest Pb blood levels of all Griffon Vultures ($n=33$) we have tested (Escobar-Gonzales *et al.* 2020). The vulture weighted 7.6 kg during tagging that was reduced to 5.88 kg postmortem, which is considered abnormal weight for a female Griffon Vulture (normal weight between 8–11 kg (Carneiro *et al.* 2016).

Morphologically, liver lesions in birds were reported as a sign of lead intoxication by Ochiai *et al.* (1993) and Pikula *et al.* (2013). The observed dystrophic changes of the reproductive organs of the vulture suggested its inactivity through the last breeding season. We hypothesise that the vulture was not able to breed because it suffered a long-term lead intoxication which affected its overall health and fertility in compliance with the findings of Vallverdú-Coll *et al.* (2016).

We registered high levels of lead in the bones (11.58 ± 1.16 mg/kg dw) of the vulture. Bone lead concentration is generally the best indicator of lead exposure over the lifetime of a bird (Franson & Pain 2011). Bone lead concentration higher than 10 mg/kg dry weight is considered as inflated and levels higher than 20 mg/kg are associated with clinical signs of acute lead intoxication (Mateo *et al.* 2003, Plaza & Lambertucci 2018).

We recorded Pb values of 4.06 ± 0.41 mg/kg dry weight, equal to 1.31 ppm wet weight (ww) after conversion, in the liver, which appeared to be consistent with the findings of LaDouceur *et al.* (2015). The author reported these levels to correspond with subclinical elevations of hepatic lead in nine cases of dead wild birds with radiographically evident gunshot projectiles in the tissues (0.29–3.84 ppm ww).

The blood Pb concentration of the vulture at the time of tagging was 384.85 ng/g, equal to 0.38 ppm and corresponded with an earlier study of U.S. Fish & Wildlife Service (1990), which found that blood lead values starting from 0.2 ppm is a threshold when toxicity symptoms begin to appear.

However, blood and liver lead concentrations, in our case, were significantly lower than that reported for Griffon Vultures suffered of acute poisoning due to ingestion of lead pellets in another study (Carneiro *et al.* 2016). In cases of chronic exposure, the highest

lead concentrations are found in bone with lower concentrations in soft tissues such as liver and blood (Franson & Pain 2011). We hypothesise that the Griffon Vulture in our study suffered a long-term chronic exposure to lead, possibly from the pellets found in its wing. Consequently, the shot was no life threatening, which allowed the bird to live long enough to accumulate lead over the threshold levels. A study of the diet of Griffon Vultures in the Eastern Rhodopes (Arkumarev *et al.* 2021) reports cause of mortality for 93 specimens used for food by the vultures, 4.3% of which were hunted or poached. Lead could also have accumulated over time as a result of ingestion of lead pellets with the food. However, blood samples from 32 other Griffon Vultures from the same population did not indicate elevated lead concentrations (Escobar-Gonzales *et al.* 2020).

Lead is a highly toxic heavy metal that acts as nonspecific poison affecting all body systems. Absorption of low concentrations may result in a wide range of sublethal effects on animals, and higher concentration may result in mortality (Franson & Pain 2011). Little is known about the origin of the lead pellets in our case, however, it is an evidence of poaching. Significant numbers of young Griffon Vultures from Eurasia migrate to the Middle East and Africa. A GPS tracked Griffon Vulture from the same colony in the Eastern Rhodopes, Bulgaria, has reached South Sudan in Africa (Arkumarev *et al.* 2019). There are estimates that at least 1.7–4.6 million birds of at least 413 species may be killed or taken illegally each year in the countries of the Middle East (Arabian Peninsula, Iran, Iraq) (Brochet *et al.* 2019). Consequently, the bird could have been shot somewhere in its wandering, overwintering or resident areas. That case stresses that lead poisoning in birds from ammunition occurs in different forms and may lead to physiological changes in the individual that affect its reproduction capacity and threaten its life.

That issue needs to be explored at a larger scale as it is currently unknown how many vultures are victims of such illegal practices. There is a need for further exploration of illegal shootings along the species' flyway and also in the residential areas in Bulgaria.

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