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*Original article*

# MONITORING OF EXPOSURE TO TOXINS IN RAPTORS IN ALBANIA

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**Summary**

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Raptors are large carnivorous birds, most well known for their scavenging nature. This study evaluates the level of exposure to toxins in raptors and vultures in Albania to assess whether existing legislation and regulations are effectively protecting vultures and other wildlife in Albania. During 2014–2018, at the Wildlife Animals Laboratory, Agriculture University of Tirana, Albania, 35 birds (*Circaetus gallicus, Gyps fulvus, Gypaetus barbatus*) were tested for exposure to toxins. The results indicated elevated blood lead levels in 65.7% of sampled raptors, while in 17.1% there was evidence of exposure to the non-steroidal anti-inflammatory drug (NSAID) diclofenac, based on visceral gout and the presence of cases with high serum uric acid concentration. Other injuries were found in 17.1% of raptors, with the most frequent being physical damage, traumatic injuries, or fractures caused by farmers, shooting, electric burn injuries from electrocution, injuries from collisions, etc. The results suggest that lead poisoning in raptors could be one of the main reasons for the observed declines in vulture populations in Albania. Furthermore, the apparent evidence for exposure to NSAIDs is concerning and requires further investigation.

**Key words**: Albania, carnivore, diclofenac, lead poisoning, uric acid, vultures

## INTRODUCTION

Over the last few decades, vulture populations have declined at catastrophic rates, especially in Asia and Africa and are now the single most threatened avian functional guild (obligate scavengers) in the world (Ogada *et al*., 2012; Buechley & Şekercioğlu, 2016). Some of the external threats that have influenced the decline in number of vultures are grouped into several categories such as: persecution, habitat destruction, decreasing food availability, dietary toxins, fishery bycatch, or stochastic events (Buechley & Seker, 2016).

For more than a century there has been information about wildlife lead poisoning through gunshots or even bullets (Calvert,

1876). The most frequent form of heavy metal toxicity in birds is due to the toxic effect of lead and is the most common poisoning to birds worldwide (Samour, 2008). Vultures are migratory birds protected by the Migratory Bird Treaty Act, State laws, and regulations and are one of the bird groups most sensitive to lead intoxication to the extent that it has been noted as a significant conservation problem for many vulture species worldwide (Golden *et al.,* 2016; Plaza & Lambertucci, 2019), threatening entire populations and compromising the success of costly conservation programs. Several toxicological studies have demonstrated that the production of small fragments of lead bullets contaminated animal carcasses and internal organs, which are too important as food sources for other animals such as scavengers (Hunt *et al.,* 2006; Knopper *et al.,* 2006; Pauli & Buskirk, 2007; Stephens *et al.,* 2009). The most endangered groups to lead exposure are predatory and scavenging birds, as when they consume carcasses embedding pieces of lead or bullet fragments as well as in the gut piles left in the field by hunters (Gill & Langelier, 1994; Locke & Thomas, 1996; Kramer & Reddig, 1997; Pain *et al.*,, 2009). According to the latest scientific research results the lead signature in vultures was closer to topsoil than to ammunition, but this similarity decreased significantly in the area with higher big game hunting activity (Arrondo *et al.,* 2020). As obligate scavengers, vultures may be at increased risk of lead exposure because lead is transported in the bloodstream and can remain in the body of vultures for days, weeks, or longer after ingestion in the acidic environment of the stomach, and absorbed through a calcium transport mechanism (Fullmer, 1991). Lead can be deposited in soft tissues (liver, kidney), bone and the growing feathers of birds, reflecting lifetime exposure (Bates *et al.*,, 1968; Pain, 1996*).* The half-life of lead in blood in birds is around two weeks and the physiological effects of lead in birds have been widely reviewed (Pain *et al.,* 2019). Several experiments and scientific studies emphasised that the toxic effects of lead in vultures are widely similar to those in all vertebrates (Eisler, 1988; Pattee & Pain, 2003; Franson & Pain, 2011). The diagnosis of lead poisoning in live birds is based on blood lead concentrations and clinical signs of poisoning whereas in dead birds, the diagnosis of lead poisoning is mostly based on tissue liver or kidney tissue lead concentrations and clinical signs of poisoning (Pain *et al.,* 2009).

This study aimed to evaluate the level of exposure to toxins in raptors in Albania and to analyse the admission causes, causes of death, and main lesions observed in reported raptors during the study.

## MATERIALS AND METHODS

Albania, a state of West Balkan, is widely known for its rich flora and fauna. Although socio-economic conditions in Albania have improved since the 1990s, there is still a lack of laws, regulations, and institutions related to the protection of the environment and wildlife. Even though in 2014 the government applied a moratorium for illegal hunting, wild carnivores are still being killed by cattlemen to protect their cattle (Anonymous, 2016). During 2014–2018, at the Wildlife Animals Laboratory, Agriculture University of Tirana, Albania, 35 individual raptors were tested for exposure to toxins – 21 *Circaetus gallicus*, 6 *Gyps fulvus*, and 8 *Gypaetus barbatus.* These cases were

mainly reported by students, environmental associations, or employees of forest directories from different regions of Albania (Fig. 1) and they were all in agony state.

Blood samples were collected from the brachial vein of raptors and stored at –80 °C until analysis, using lithium heparin blood tubes. The collected blood samples were centrifuged at 1200 rpm for 15 min in a refrigerated centrifuge (4 °C) in order to separate the serum from the supernatant. Blood samples were analysed for lead concentration at the Wildlife Animal Laboratory, Agriculture University of Tirana, in collaboration with the other laboratory part of the Balkan Anti Poisoning Project, Albania: Anti poisoning Centre Supported by VCF (Vulture Conservation Foundation) using graphite atomic absorption spectrophotometry. Using this methodology, blood lead concentrations ≤10 µg/dL were consistent in the same level to exposure environmental background sources of lead. Concentrations >20 µg/dL were equivalent to sublethal exposure, indicating increased exposure of lead, and values >50 µg/dL: to lethal lead exposure (Franson & Pain, 2011). The detection of uric acid in serum samples was done with commercial kit (ACE TM Uric Acid Reagent).


**Fig. 1.** Map of raptor samples in different regions of Albania.

RESULTS

A total of 35 raptors were analysed at the Wildlife Animals Laboratory for different poisonings. The results indicated the presence of lead poisoning in 65.7% in seven districts of Albania. In our study the elevated blood lead concentrations were present in 23/35 raptors (Table 1).

Table 2 shows the data obtained from our study for the detection of lead concentration in blood samples. The mean blood lead concentration was 27.2 µg/dL (range: 20–30 µg/dL), while in 17.1% there was evidence of exposure to the non-steroidal anti-inflammatory drug (NSAID) diclofenac, based on visceral gout and the presence of cases with high serum uric acid concentration.

Other causes of injuries were identified in 6 birds (17.1%) – Table 3. Factors associated with injury of vultures were mainly physical damage, traumatic injuries, or fractures caused by farmers as they always treat vultures as predators of their livestock, shooting, injuries from electric

**Table 1.** The overall results of vultures study in Albania

| Species name | Number | Increased blood lead concentration | Evidence for diclofenac exposure | Other causes of death |
|---|---|---|---|---|
| *Circaetus gallicus* | 21 | 15/21 = 71.4% | 2/21 = 9.5% | 4/21 = 19.0% |
| *Gyps fulvus* | 6 | 3/6 = 50% | 1/6 = 16.6% | 2/6 = 33.3% |
| *Gypaetus barbatus* | 8 | 5/8 = 62.5% | 3/8 = 37.5% | – |
| Total | 35 | 23/35 = 65.71% | 6/35 = 17.1% | 6/35 = 17.1% |

**Table 2.** Lead concentration value in 23 blood samples of raptors

| Region/ Location | Species | Lead concentration (µg/dL) | Mean lead concentrations |
|---|---|---|---|
| Vlorë | [6]*Circaetus gallicus* | 30 µg/dL, 26 µg/dL, 25 µg/dL, 30 µg/dL, 30 µg/dL, 28 µg/dL | 28.1 µg/dL |
| Gjirokastër | [3]*Circaetus gallicus*<br>[1]*Gyps fulvus* | 21 µg/dL, 27 µg/dL, 30 µg/dL, 22 µg/dL | 25 µg/dL |
| Korçë | [1]*Gypaetus barbatus*<br>[1]*Circaetus gallicus* | 21 µg/dL, 30 µg/dL | 25.5 µg/dL |
| Lushnje | [1]*Circaetus gallicus*<br>[1]*Gyps fulvus* | 30 µg/dL, 25 µg/dL | 27.5 µg/dL |
| Fier | [2]*Gypaetus barbatus*<br>[1]*Circaetus gallicus*<br>[1]*Gyps fulvus* | 30 µg/dL, 30 µg/dL, 24 µg/dL, 30 µg/dL | 28.5 µg/dL |
| Shkodër | [1]*Gypaetus barbatus*<br>[1]*Circaetus gallicus* | 30 µg/dL, 28 µg/dL | 29 µg/dL |
| Dibër | [1]*Gypaetus barbatus*<br>[3]*Circaetus gallicus* | 29 µg/dL, 30 µg/dL, 26 µg/dL, 24 µg/dL | 27.25 µg/dL |
| | | Total mean value | 27.2 µg/dL |

**Table 3**. Results for blood lead and uric acid levels and other causes of death of 35 raptors in 7 regions of Albania

| Region/ Location | Species | Increased blood lead concentration | Evidence for diclofenac exposure | Other causes of death | | | |
|---|---|---|---|---|---|---|---|
| | | | | Physical damage, traumatic injuries | Shooting, fractures caused by farmers | Electric burn injuries from electrocution | Injuries from collisions |
| Vlorë (n=6) | [6]*Circaetus gallicus* | [5]*Circaetus gallicus* | [1]*Circaetus gallicus* | | | | |
| Gjirokastër (n=8) | [7]*Circaetus gallicus*<br>[1]*Gyps fulvus* | [3]*Circaetus gallicus*<br>[1]*Gyps fulvus* | [1]*Circaetus gallicus* | *Circaetus gallicus* | *Circaetus gallicus* | *Circaetus gallicus* | |
| Korçë (n=2) | [1]*Gypaetus barbatus*<br>[1]*Circaetus gallicus* | [1]*Gypaetus barbatus*<br>[1]*Circaetus gallicus* | | | | | |
| Lushnje (n=3) | [1]*Gypaetus barbatus*<br>[1]*Circaetus gallicus*<br>[1]*Gyps fulvus* | [1]*Circaetus gallicus*<br>[1]*Gyps fulvus* | [1]*Gypaetus barbatus* | | | | |
| Fier (n=4) | [2]*Gypaetus barbatus*<br>[1]*Circaetus gallicus*<br>[1]*Gyps fulvus* | [2]*Gypaetus barbatus*<br>[1]*Circaetus gallicus*<br>[1]*Gyps fulvus* | | | | | |
| Shkodër (n=5) | [2]*Gypaetus barbatus*<br>[1]*Circaetus gallicus*<br>[2]*Gyps fulvus* | [1]*Gypaetus barbatus*<br>[1]*Circaetus gallicus* | [1]*Gypaetus barbatus* | | | | [2]*Gyps fulvus* |
| Dibër (n=7) | [2]*Gypaetus barbatus*<br>[4]*Circaetus gallicus*<br>[1]*Gyps fulvus* | [1]*Gypaetus barbatus*<br>[3]*Circaetus gallicus* | [1]*Gypaetus barbatus*<br>[1]*Gyps fulvus* | | [1]*Circaetus gallicus* | | |

burns, electrocution, injuries from collisions, etc.

## DISCUSSION

Presented results are consistent with exposure to a point source of concentrated lead in several mined areas since 1945–1991 that Albania has gone through. Due to the lack of cleaning of these mined areas for so many years since that period, these areas became the main point of lead poisoning for many aquatic and terrestrial vertebrate organisms. Nowadays, these toxic waste remains are discharged into aquatic environments, increasing the number of affected organisms. Some factors such as length and duration of exposure and size of the original dose are considered too important in order to create a relationship between an individual's condition and its blood lead concentration (Pattee *et al*., 2006). When the levels of lead are quite high in these animals, they can cause significant secondary lead poisoning in predators or scavengers. Terrestrial and aquatic birds are at high risk of lead poisoning from ammunition. This is just one of the many reasons that nowadays there is an attempt to replace lead in ammunition with non-toxic alternatives. Other factors that may have contributed to the increasing number of raptors including vultures poisoning by lead in recent years in Albania, may be the presence of unclean military areas or the presence of car battery accumulations in the latest years in Albania.

Nowadays it is difficult and costly to clean the environment in Albania after the deposition of lead ammunition. It is thought that this may be a major factor for increasing the number of lead-infected raptors in Albania after consumption of poisoned carcasses before their death in rural areas is getting problematic. The bioaccumulation process in some edaphic organisms, comes as a result of the bioavailability of lead from several sources of ammunition (Migliorini *et al.,* 2004), and puts at risk vegetation, invertebrates, and other organisms (Bennett *et al.,* 2007). The concentrations of lead in raptors were high which supports the data about problems with body contamination with this heavy metal. Our results emphasised the idea that the main cause of toxic lead concentration in vultures is ammunition (García-Fernández, 2014, Krone, 2018; Pain *et al.,* 2019).

Strong measures must be undertaken by the institutional authorities in Albania. The loss of millions of vultures over the last decade has had major ecological consequences across continents that pose a potential threat to human health. Sufficient data are available for making approximate estimates of annual numbers of deaths caused by lead poisoning in wildfowl. These are necessarily imprecise as many external factors affect lead-poisoning mortality from year to year, notably food availability and weather (Pattee & Pain, 2003). In Albania, the hunting activity has been one of the most favorite activities of people over the years, especially after the end of the communist period.

Even though in 2014 the government applied a Moratorium for illegal hunting, wild carnivores are still being killed by cattlemen to protect their cattle. According to Table 1, six serum samples (17.1%) from *Circaetus gallicus, Gypaetus barbatus,* and *Gyps fulvus* were suspected cases for diclofenac poisoning based on the sign visceral gout and the presence of uric acid in concentration serum. In the Albanian domestic livestock, diclofenac is widely used for the

symptomatic treatment and management of inflammation, fever, pain associated with disease or injury (Kapsalis, 2019). Cattle treated before their death with diclofenac serve as carcasses for vultures that are exposed to this drug. Environmental protection employees need to take remedial initiatives to guide and inform this community in order to preserve the number of these very important birds in ecology such as vultures (Dupont *et al.,* 2012; O'Rourke, 2014; Morales-Reyes *et al.,* 2015; Morales-Reyes *et al.,* 2017). They should also inform the farmers to limit the direct contacts of vultures with livestock treated with different drugs. One possible way to reduce this direct contact of vultures with sick animals is keeping them indoors for a short period until their recovery. If non-steroidal anti-inflammatory drugs (NSAIDs) were identified as effective in treating various cattle diseases that are relatively non-toxic to vultures, it would be a good alternative to eliminate diclofenac from the food of vultures. Recently, based on some scientific studies, they have reported some facts for replacement of diclofenac NSAID with meloxicam as they have demonstrated that meloxicam is much less toxic than diclofenac. There is also no evidence that meloxicam can cause death or even elevation of serum uric acid concentrations.

## CONCLUSION

Lead poisoning is the main reason for the observed deaths in vultures and strong measures must be undertaken by the institutional authorities in Albania to limit the use of lead ammunition. The general public, mainly farmers, should be made aware of the natural behaviour of vultures. As the Environmental and Animal Protection Organisations in Albania have expressed concern about the negative effects of diclofenac, the results of our study further strengthen this concern. Considering the drastic decline in vulture population, the responsible Albanian Authorities should undertake action to reduce the use of diclofenac in agriculture by substituting it with a NSAID which is less toxic and associated with a low percentage of death in vultures population.

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