



## Levels of blood lead in Griffon vultures from a Wildlife Rehabilitation Center in Spain



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### ABSTRACT

Lead is considered a highly toxic contaminant with important impacts to bird wildlife. Griffon vultures (*Gyps fulvus*) are a sensitive indicator of the level of environmental contamination due to their position at the top of the food chain and their dependence on human activities. The aim of this study was to assess susceptibility to lead intoxication in Griffon vultures admitted to Wildlife Rehabilitation Centers (WRC), measuring blood lead levels and determining if blood lead concentrations are related to clinical signs, hematological, biochemical or radiographic findings. Also, the influence of age, gender, body condition, season and primary cause of admission were evaluated. This study was realized in all Griffon vultures admitted during a period of one year in the Rehabilitation Center GREFA. Blood lead levels are measured by using anodic stripping voltammetry. In Griffon vultures, we observed that 26% of the analyzed birds presented lead levels above 20 µg/dL with 74% below 20 µg/dL ( $[Pb]_{<20} = 9.34 \pm 5.60 \mu\text{g/dL}$ ). In our study, statistically significant differences were found for lead according to sex, season of admission to the center and body condition. A negative correlation was found between levels of metal and hematocrit. No association was found between clinical signs and blood lead levels in Griffon vultures, except for digestive signs as stasis and weight loss. On numerous occasions, the intoxication in this specie is related to ingestion of lead ammunition; however, we have not detected radiographic lead in our vultures. Compared with other studies, we generally found low levels of lead in blood of Griffon vultures but the blood of all birds admitted to WRC presented detectable lead concentrations. This species apparently presents a higher sensibility to the toxic effects of this metal than that described by other authors. It has been observed that there is some evidence that suggests that subclinical levels of lead could be related with a predisposition to injury or diseases, even though these birds might be admitted for other causes. The detection of levels of blood lead in animals that are admitted to a recovery center will give valuable information which could be used to monitor spatial and temporal variations and provide a clearer picture of temporal levels of this contaminant in this emblematic avian specie.

### 1. Introduction

Population surveys of vultures in the world have indicated a marked decrease in numbers of several species. The causes for the population decline remains unknown, even though numerous factors are likely implicated. It has been shown that environmental contaminants such as lead are involved in this problem (Pain et al., 2005). Although the negative consequences of lead to avian scavengers are well known, there are relatively limited available data on lead exposure across avian scavengers (Behmke et al., 2015; García Fernández et al., 1995; García Fernández et al., 2005; Carneiro et al., 2015; Rodríguez Ramos et al., 2008; Espín et al., 2014). Although, Griffon vultures are currently evaluated as Least Concern on the IUCN Red list of threatened Species

(IUNC, 2015), a negative impact on this specie has been observed. The Griffon vulture, a long-lived diurnal scavenger belonging to the family Accipitridae (Order Accipitriformes) and mostly resident, was the species selected for this study. This specie is a sensitive indicator of the level of environmental contamination due to their position at the top of the food chain and their dependence on human activities.

In previous studies, Carneiro et al. (2015) investigated heavy metal concentrations of 3 compounds in Griffon vultures from the Iberian Peninsula. They reported that cadmium and mercury were not detected in most samples (98.3% and 95%, respectively), while lead (Pb) was detected in all birds. The prevalence and the prognostic of this intoxication make the time required to confirm a lead intoxication paramount. Initiating treatment would be, in general, a crucial factor to

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avoid mortality and to improve the chances of survival of the bird. In many Wildlife Rehabilitation Centers (WRC), this intoxication is identified by clinical signs and radiographic, hematology and biochemical analysis. Analysis of blood by spectrometry is a common method for lead detection in diagnostic laboratories. This method presents a high selectivity and sensibility for this metal; however, it's costly and diagnosis is delayed due to the time it takes to ship and to process the samples in the laboratories (Redig, 2008; Bischoff et al., 2010). For these reasons, the use of a method based on electrochemistry, Anodic Stripping Voltammetry (ASV), permits a rapid determination of lead in blood samples.

Exposure to low concentrations of lead can induce neurological effects similar to those described in human beings. There is evidence that low-level Pb exposure, although not causing the clinical signs of classical Pb poisoning, may nevertheless have subtle detrimental effects on normal behavior and cognitive function (Carneiro et al., 2014) and these birds could be admitted in the WRC for other causes such as electrocution, vehicle collision, ... In our WRC (GREFA), when these problems affect young birds, often this is justified by a poor ability to find food, which causes weakness and increased likelihood of suffer trauma. For these reasons, the objectives of this study were to assess susceptibility to lead intoxication in griffon vulture (*Gyps fulvus*) admitted to WRC, measuring blood lead levels and determining if lead blood concentrations are related to clinical signs, hematological, biochemical or radiographic findings. Also, the influence of age, gender, body condition, season and primary cause of admission were evaluated.

## 2. Materials and methods

This study was realized in all Griffon vultures (*Gyps fulvus*) admitted during a period of one year (March 2014 to March 2015) in the Rehabilitation Center GREFA of Spain. The birds (n = 32; 19 males, 11 females and 2 unknown sex) came from different areas of Spain and were admitted to the Rehabilitation Center due to various causes, with the level of lead being determined in all animals included in this study. From each animal, the body mass, age group (chicken, juvenile or adult) and sex (where possible, using a PCR method or necropsy) were determined. The ages of Griffon vultures admitted to WRC were mostly juveniles (80 days-2 years old; n = 26). Also, 4 adults and 2 chickens were studied. The study was carried out in accordance with ethical considerations on animal welfare.

A complete clinical evaluation was performed of all birds (general body conformation, posture attitude, stimulus response, respiration and exploration of feathers, skin, beak, eyes, cere, nares, oral cavity, bones, muscles, abdomen and ventus). The observation of breast muscle was used to determine the nutritional state (body condition score based on a score of 1–5, with 1 being emaciated and 5 being obese). The following clinical signs were assessed in all birds included in the study: neurological, digestive, hematologic and behavioral. Blood samples were collected via venipuncture from the ulnar or metatarsal veins using disposable 23-gauge needles and 2 mL plastic syringes. A drop of whole blood was used to perform a blood smear, by means of the slide on slide technique (Campbell, 1995), and a small amount was immediately withdrawn into a heparinized microcapillary tube for packed cell volume (PCV) determination. The remaining sample was transferred into a commercially available blood tube with ethylene diamine tetra-acetic acid (EDTA, 1.5 mg/mL of blood), for hematological analysis and a blood tube with heparin lithium for biochemistry and lead detection. Samples were refrigerated at 4–6 °C and tested within 24 h of collection. Plasma biochemistry was performed using a Vet-Scan VS2 Analyzer, Abaxis, Union City, CA, USA) with a rotor (Avian/Reptilian Profile Plus rotor, Abaxis) and the plasma enzymes activities analyzed were aspartate aminotransferase (AST: EC2.6.1.1), and creatine phosphokinase (CPK, EC 2.7.3.2). The plasma constituents analyzed were: albumin, globulin, total protein, uric acid,

calcium, phosphorus, sodium and glucose. The presence of lead in the digestive tract or in other tissues of birds were assessed using the radiographic technique (INTECH S.L., Futura 10. Cassette REGIU cassette RC-300, Konica Minolta). The radiography was performed in ventrolateral position. The presence of parasitic infection was evaluated using a coprological and hematological analysis and an external inspection.

Blood lead levels were measured using an electrochemical system (LeadCare II Blood Lead Testing System, ESA Inc, Chelmsford, MA USA) employing a technique called anodic stripping voltammetry (Yantasee et al., 2007). The analytical reporting range is 3–65 µg/dL (0.14–3.14 µmol/L) with a measuring time of 3 min. All samples were analyzed within 24 h of blood extraction.

Thirty two birds were included in this study, weighting  $6.78 \pm 1.49$  kg (2.9–9.1 kg). Two animals were excluded from statistical analysis. One bird was an adult admitted by WRC in shock and the other one suffered a collision, which is why samples were not obtained. Both animals presented severe clinical signs and they died within a few hours. The bird, which presented the highest value of lead concentration (> 65 µg/dL), was not included in the statistical analysis due to this value was higher than the superior limit of quantification of the method. Unfortunately, this sample could not be sent to a reference laboratory.

The descriptive and inferential statistic of the data was performed using SPSS 22 software package (IBM). The normality of data was analyzed by Shapiro-Wilk test. Data were analyzed using an analysis of variance (ANOVA) with *post hoc* Bonferroni test. Spearman's rho test was used to detect the strength of association between lead concentrations with the other quantitative variables studied. A statistical significance level of  $p < 0.05$  was used for null hypothesis rejection.

## 3. Results

In Griffon vultures, we observed that 26% of the analyzed birds presented lead levels above 20 µg/dL with 74% below 20 µg/dL ([Pb] =  $9.34 \pm 5.60$  µg/dL). A global mean value of  $15.78 \pm 13.32$  µg/dL (median = 12.90; geometric mean = 11.22 µg/dL; n = 31) was obtained, taking into account that the bird with [Pb] > 65 µg/dL was not included in this estimate. In our study, statistically significant differences were found for lead according to gender, season of admission to the center and body condition. The most vultures of [Pb] > 20 µg/dL had a body condition score of 1–2 (Fig. 1, Tables 1, 2). Bird with lead blood levels > 65 µg/dL was not included in the statistical analysis, but it presented a body condition score of 3 with nervous and digestive signs. In our study (Fig. 2, Tables 1, 2), we observed a lower value of lead blood levels in female as opposed to male birds ( $p = 0.016$ ). Statistically differences were found for lead according to season of admission; finding differences between spring and summer or autumn (Fig. 3, Tables 1, 2). The highest lead concentrations were obtained in autumn. No difference between age groups was found in our study ( $p > 0.05$ ).

The biochemical and hematological values found in *Gyps fulvus* are shown in Tables 1, 2. There is a correlation between Pb and PCV % (rho de spearman = -0.506;  $p = 0.004$ ). Close to ninety percent of birds with background exposure to lead had a PCV above 40%, being within the reference range of this specie (Polo et al., 1992). However, this percentage was reduced to 30% when the exposure was greater than 20 µg/dL (Fig. 4). In griffon vultures, we observed that PCV shown statistical differences among the seasons of admission and between the genders. There is a negative correlation between Pb and hemoglobin (Rho de spearman = -0.605;  $p = 0.001$ ). There are correlation between lead and Ca, P or globulin. Statistical differences were found to Ca (body condition and season), P (body condition), globulin (body condition, season and gender) and protein (gender), but the majority of these values were within the physiological range. Also, the values obtained for these biochemical parameters were not related with the

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