High Levels of Blood Lead in Griffon Vultures (*Gyps fulvus*) from Cazorla Natural Park (Southern Spain)

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ABSTRACT: The blood lead of 23 griffon vultures (*Gyps fulvus*) trapped in 2003 was analyzed in order to evaluate exposure to lead in the vulture population of Cazorla Natural Park (in southern Spain). In 2001 the use of leaded gasoline in vehicles was banned in the European Union; however, lead ammunition is still used in Spain in big-game hunting for red deer, fallow deer, mouflon, and wild boar, which are ingested by vultures from September to March. The mean concentration of lead in blood was 43.07 ± 31.96 μg/dL with a range of 17.39–144.80 μg/dL. Only two vultures had lead levels below 20 μg/dL, and two others had blood lead concentrations close to 150 μg/dL. In view of the results, we think the population of vultures from Cazorla Natural Park is suffering subclinical exposure to lead, with some individuals exposed to high toxicity risk. We concluded that ingestion of lead in the metallic form alone is sufficient to produce these blood lead concentrations, and we recommend the prohibition of lead ammunition for big-game hunting in order to preserve the vulture population. Environ Toxicol 20: 459–463, 2005.

Keywords: griffon vulture; *Gyps fulvus*; blood lead; shotgun

INTRODUCTION

Lead is one of the most toxic heavy metals to living beings. Its environmental ubiquity and persistence and its accumulation in organisms and biomagnification throughout the trophic chain imply continuous exposure. Acute poisoning by this metal can cause mortality, and chronic exposure to lead can indirectly affect avian populations by altering reproductive success, behavior, immune response, and physiology (Mazliah et al., 1989; Burger and Gochfeld, 2000; Fair and Ricklefs, 2002) and therefore may cause population declines in sensitive or vulnerable species.

Wild birds may sometimes be exposed to high metal levels, for example, at waste disposal sites (such as at Aznalcollar, Spain, in 1998) or through the ingestion of lead-shot pellets, weights, and game meat impacted with fragments of lead ammunition. Such acute poisoning is easily diagnosed, although long-term effects are difficult to assess. Other sources of lead (such as lead-gasoline exposure or mining and smelting activities, which are related to lesser blood lead concentrations) are less common and rarely provoke clinical symptoms or death (Garcia-Fernandez et al., 2003).

Lead concentration in blood is a good indicator of recent exposure, whereas chronic exposure can be estimated by

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measuring accumulated concentrations in tissue (bone) samples, when available. The association of blood lead concentration with several physiological and pathological effects is well established in *Falconiformes* (Franson, 1996), but is not as well established in vultures, except for some data obtained by Carpenter et al. (2003), Donazar et al. (2002), and Platt et al. (1999). The present study was carried out on a species of vulture (*Gyps fulvus*) that inhabits a Mediterranean forest with no specific sources of heavy-metal pollution. Blood lead concentrations were analyzed in 23 individuals from Cazorla Natural Park, an important zone where game hunting is one of the most important sources of income.

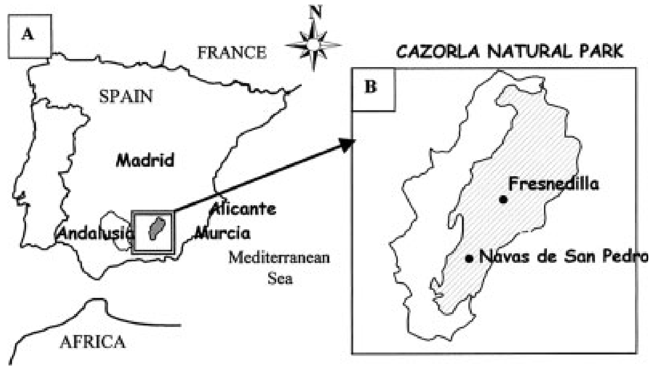

Fig. 1. Maps showing the geographical location of Cazorla Natural Park (Scale A = 1:16,800,000).

MATERIALS AND METHODS

Area and Specie of Study

The study area, covering about 214,000 ha, is a mountainous area of Mediterranean forest (Fig. 1) with some marginal cultivation of olive trees and highlands with extensive grazing of sheep and cattle, surrounded by crops for dry farming. It is far from any urban, industrial, or mining zones. The climate is typically Mediterranean, with cold winters and warm summers. The nearest industrial area, urban area (population > 10,000 h), and highway are 100, 10, and 80 Km away, respectively. Since 1998 several regulations have been approved by the European Union (EU) and Spain that progressively restricted and, in 2001, finally banned the use of leaded gasoline in vehicles (Directive 98/79/CE; Royal Decree 785/2001). There were 250 pairs of griffon vultures in the area in 2003. Their diet from September to March consists of fallow deer (*Dama dama*), red deer (*Cervus elaphus*), mouflon (*Ovis mousimon*), and wild boar (*Sus scofra*). The rest of the year they live on carcasses of sheep, goats, and cows.

Collection of Samples

In June 2003, 23 griffon vultures (*Gyps fulvus*) from two zones (Fresnedilla and Navas de San Pedro) of Cazorla Natural Park were caught with a canyon net using a carcass of roe deer as bait. To avoid stress in the vultures, careful steps were taken. Blood samples were taken by the veterinarian of the Santa Faz Wildlife Recovery Center (Alicante, Spain), who also evaluated the health status of the captured vultures. Finally, they were liberated. Blood samples from the vultures (2.0 mL) were taken by puncturing the radial vein using a hypodermic needle and syringe. These were taken immediately to the laboratory in refrigerated conditions and were frozen at −40°C until processing.

Preparation of Samples for Analysis of Lead

Samples were prepared for analysis by anodic stripping voltammetry (ASV). After eliminating all organic impurities that might interfere with the results, complete digestion was ensured by using high-temperature digestion with a mixture of acids following the method described by Garcia-Fernandez et al. (1995). All the reagents used were of Suprapur® quality from Merck (Darmstadt, Germany). The quartz tubes used for the wet digestion were previously washed with 2% nitric acid for 48 h and then rinsed twice with tetradistilled water and dried in an oven at 100°C. A volume of 0.2 mL of whole blood was placed in a quartz digestion tube, to which 0.5 mL of an acid mixture (nitric/perchloric/sulfuric, 8:8:1) was added. The sample was then submitted to a progressive thermal treatment and, once dried, was left to cool. Tetradistilled purified water was added and transferred to the measuring vessel, adjusting the final volume to 10 mL.

Analysis of Lead

Prior to ASV, 100 μL of hydrochloric acid was added to the measuring vessel as an electrolyte support. The pH of the final solution was between 1 and 2. The anodic stripping voltammeter (VA-646 processor and VA-647 workstation, Methrom, Switzerland) used was equipped with three standard electrodes: working electrode (hanging mercury drop), reference electrode (Ag/AgCl, KCl 3 mol/L), and auxiliary electrode (platinum). We used the differential pulse normal technique with an electrolysis time of 180 s and a modulation amplitude of 50 mV. The concentration of lead in the digested sample was calculated after twice adding dilutions prepared from standard solutions of lead (Sigma, St. Louis, MO, USA). The detection limit was 0.15 μg/dL, and the repeatability, which was determined by analyzing 10 identical samples of reconstituted lyophilized blood (European Union Reference Standards) CRM195, was 96.5% ± 1.2% (Garcia-Fernandez et al., 1995).

Statistical Analysis

Statistical analysis of the data was performed using SPSS v10.0 statistical software (SPSS Inc., 1989–1999). Comparisons between sampling zones and among age groups were

TABLE I. Statistical data for body weight, tarsus length, and blood lead concentration of the 23 vultures sampled in Cazorla Natural Park in June 2003

	N	Minimum	Maximum	Mean	Standard Deviation
Body weight (kg)	22	7.25	9.50	8.35	0.55
Tarsus length (cm)	21	109	160	119.62	12.93
Blood lead (μg/dL)	23	17.39	144.80	43.07	31.96

analyzed using the nonparametric Mann–Whitney and Kruskal–Wallis tests, respectively. All vultures sampled in Fresnedilla ($n = 6$) were subadult, and therefore the mean of blood lead concentrations was compared with the corresponding subadults from Navas de San Pedro ($n = 5$). The level of significance was set at $\alpha = 0.05$. Spearman's rank nonparametric correlation test was applied to examine any relationships between lead concentrations in the blood of the vultures, body weight, and tarsus length.

RESULTS AND DISCUSSION

Mean comparison tests did not show significant differences between sampling zones (body weight, $p = 0.295$; tarsus length, $p = 0.346$; lead concentration, $p = 0.361$) or among age groups (body weight, $p = 0.230$; tarsus length, $p = 0.811$; lead concentration, $p = 0.484$), and therefore all vultures were considered as only one population with respect to lead exposure. No significant results were obtained from the correlation test. Data on body weight, tarsus length, and blood lead concentrations are shown in Table I.

Knowledge of blood lead concentrations associated with different kinds of lead exposure and toxicity is an important prerequisite when blood lead concentration is being used to assess the risk of lead exposure to wild populations. We have not found references on blood lead levels in griffon vultures, and therefore we sought data on other wild-bird species in order to discuss our results. Data from vulture species other than the griffon also are scarce, and experimental studies have been performed only on turkey vultures (*Cathartes aura*; Reiser and Temple, 1981; Carpenter et al., 2003). However, more information is available on other raptors, and relevant data were offered on Falconiformes by Franson (1996), who reviewed and classified the different types of lead exposure based on blood lead concentrations (Table II). Comparison of the results offered by Carpenter et al. (2003) and Franson (1996) showed that the turkey vulture appears to be relatively tolerant to lead poisoning in comparison to other raptor species.

In a recent work on forest raptors [the booted eagle (*Hieraaetus pennatus*), the European buzzard (*Buteo buteo*), and the goshawk (*Accipiter gentilis*)] that inhabit a Mediterranean ecosystem far from heavy-metal sources, we observed decreased blood δ-ALAD activity to be related to blood lead concentrations below 5 μg/dL. In that study blood lead concentrations ranged between 1.1 and 12.0 μg/dL, with a mean of 3 μg/dL (Martínez-López et al., 2004). Similar low values were found in a previous study on diurnal and nocturnal raptors in which a probable decrease of δ-ALAD activity was suggested but no reproductive and behavioral effects were related (Garcia-Fernandez et al., 1997). The mean concentration of lead in the blood of the griffon vultures in the present study was 5–16 times higher than those mentioned in these previous studies.

Only two vultures in this study had lead levels below, but close to, 20 μg/dL, mentioned by Franson (1996) as the minimum blood lead level necessary in Falconiformes for considering physiological effects (Table II). The mean blood lead concentration in griffon vultures (43.07 μg/dL) was twofold greater than this minimum level and fourfold higher than the mean concentrations in forest raptors (Martínez-López et al., 2004). Moreover, two other vultures had blood lead concentrations (132 and 144 μg/dL) close to 150 μg/dL, considered by Franson (1996) as the threshold value in individuals with probable clinical symptoms (Table II).

In view of these results, it is probable that exposure in this vulture population is far from a typical background level of environmental exposure to lead. Moreover, most

TABLE II. Interpretation of blood lead concentrations in Falconiformes (Franson, 1996)

Exposure	Blood Lead (μg/dL)	Observations
Subclinical	20–50	Physiological effects, first δ-ALAD[a] depression
Toxic (clinical)	> 100	Clinical signs such as muscle wasting, weakness, anemia, weight loss, green diarrhea, and muscular incoordination
Compatible with death	> 500	From mortality reports

[a] δ-Aminolevulinic acid dehydratase activity.

individuals are likely to have suffered subclinical exposure to lead, but few individuals are likely to be suffering from clinical poisoning.

Because there are no sources of lead from industries, cities, or mining close to Cazorla Natural Park, lead exposure in these vultures is not likely to be related to environmental pollution of lead in the atmosphere or soil. On the other hand, the blood lead levels were higher than those found in other species living near highways or mining zones (Garcia-Fernandez et al., 2003). Also, it is improbable that these lead levels resulted from ingestion of animal viscera into which environmental lead was biologically incorporated. We do not have data on other species from Cazorla Natural Park; however, in a previous study on red deer and wild boar from Sierra Morena (150 km from Cazorla), we detected lead concentrations in the liver and kidney below 1 mg/kg in animals sampled far from a mining zone (Santiago et al., 1998). It is probable that the ingestion of lead in metallic form alone is able to provoke these blood lead concentrations. In Spain the use of lead ammunition has been restricted only in wetlands included on Ramsar's list because of the well-known risk to waterfowl (Royal Decree 581/2001). However, lead ammunition is still frequently used in big- and small-game hunting. In Cazorla Natural Park big-game hunting is allowed and regulated from September until March for controlling the red deer, fallow deer, and wild boar populations. Carcasses of shot animals serve as food for the vultures. It is very probable that ingestion of game meat impacted with fragments of lead shot is the major cause of the high blood lead concentrations obtained in this study.

Acute Pb poisoning has been recognized as one of the most important causes of mortality in raptors (Pattee and Hennes, 1983; Wiemeyer et al., 1988) and therefore a potential cause of decline in raptor populations. In the Ebro delta, Mateo et al. (1999) found that 2.5% of marsh harriers (*Circus aeruginosus*) had a blood lead concentration higher than 100 μg/dL, the threshold level indicative of Pb poisoning in this species. Mateo et al. (1999) also suggested that one bird with a blood lead concentration of 80 μg/dL that had an open abscess in the oral cavity could have been suffering immunosuppressive effects from lead exposure. In our study a substantial percentage of the vultures trapped had blood lead levels higher than 70 μg/dL (13%) and 100 μg/dL (8.7%). Other effects related to lower blood lead concentrations have been described, such as reduced reproductive success and embryonic mortality (Buerger et al., 1986), poor body condition (Mateo et al., 1997), and breeding failure (Newton, 1979). We do not have information about the susceptibility or tolerance of vultures to lead, and therefore, in view of these results, we must consider the potential risk of lead poisoning in the vulture population studied. Moreover, we recommend the prohibition of lead ammunition in big-game hunting activity in order to preserve the population of vultures.

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