Assessing the risk of lead exposure for the conservation of the endangered Pyrenean bearded vulture (Gypaetus barbatus) population

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A B S T R A C T

Acute and chronic lead (Pb) poisoning have been recognized as some of the most important causes of mortality for raptors worldwide. We simultaneously examined the recent, medium-term and long-term lead exposure of the endangered bearded vulture (Gypaetus barbatus) from the Pyrenees (northern Spain and southern France). One hundred and one blood samples from 87 captured individuals and tissue samples (liver and bone) from a further 43 dead individuals were analyzed for lead residues. The majority of individuals examined had very low lead concentrations in blood, liver and bone. However, two individuals showed elevated blood Pb levels, two individuals showed liver lead concentrations indicative of excessive lead exposure and one individual showed bone lead levels indicative of chronic lead poisoning, suggesting that the Pyrenean population is not free from the risk of poisoning. We found that Pb exposure was significantly higher in adult individuals as well as in the northern (France) and eastern (Catalonia) range of their distribution. These differences could be related to different feeding habits between age classes (pre-adults are more linked to supplementary feeding sites) and differences in hunting practices between regions (in some regions, carcasses and offal of game animals are not retrieved). Blood, liver and bone lead levels found were slightly higher during the hunting season than outside of the hunting season. Lead presents an unnecessary threat to adult birds and the only way to remove this risk is to ban all hunting with lead within the range of distribution of the endangered bearded vulture. Acute and chronic lead poisoning should be considered in differential diagnosis in any diseased or injured wild bearded vulture, especially subadult and adult individuals, and the potential risk of Pb poisoning should be considered in future reintroduction programs.

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1. Introduction

Lead (Pb) is a highly toxic heavy metal that acts as a non-specific poison affecting all body systems (Pain, 1996). Detrimental health effects of Pb toxicity on waterfowl have long been recognized as an environmental problem worldwide (Wayland et al., 2003), but Pb poisoning also affects non-waterfowl species, a key group belonging to Falconiformes (see Fisher et al., 2006 for a general review). Pb poisoning is the most frequent type of heavy metal poisoning in birds of prey (Franson, 1996; Pain et al., 2005). The primary source of Pb in poisoned raptors is assumed to be the consumption of prey containing Pb shot or bullet fragments (Kendall et al., 1996; Mateo et al., 1999; Clark and Scheuhammer, 2003). Although some raptors may regurgitate shot effectively (Patte et al., 1981), part of the Pb contained in shot or bullet fragments is rapidly dissolved and absorbed after ingestion due to their low stomach pH, and often results in acute death (Fisher et al., 2006; García-Fernández et al., 2005). Sublethal exposure is also of concern since it may predispose birds to other causes of death, but it is less commonly studied (Wayland et al., 2003; Mateo et al., 2003; Pain et al., 2007). Both acute and chronic Pb poisoning have been relevant in the decline (Wiemeyer et al., 1988; Snyder, 2008) and successful reintroduction of the Californian condor (Gymnogyps californianus) (Cade, 2007; Hall et al., 2007; Green et al., 2008) and it is an important threat for several other critically endangered raptors worldwide (Kramer and Redig, 1997; Kim et al., 1999; Kenntner et al., 2001; Clark and Scheuhammer, 2005; Pain et al., 2005).

The bearded vulture or lammergeier (Gypaetus barbatus) is endangered in Europe and is a species of conservation concern (Annex I, EU Wild Birds Directive 79/409/EEC, Appendix II of the Bern Convention, Bonn Convention and CITES), with around 150 breeding territories in the European Union in 2007. In Europe, intentional and unintentional poisoning have been considered the most important cause of mortality (Margalida et al., 2008).
In South Africa, the decline in the distribution and numbers of the bearded vulture population have been attributed to the poisoning and a reduction in the food supply (Brown, 1997). The knowledge about causes of death, illness and injury can aid and facilitate the application of management measures to improve conservation of this endangered species (Margalida et al., 2008). In a recent study (Oro et al., 2008), it was demonstrated that the bearded vulture population is quite sensitive and vulnerable to decreases in adult survival and the recent trends of this age class in the Pyrenean range indicate that mortality, including poisoning, has increased over the last few years. However, to date, no attempts were made to determine if the bearded vulture may be at particular risk for ingestion of fragments of Pb ammunition embedded in dead game animals and to what extent this risk exists.

In the present study, we report the results of a survey from the period 1990–2009 in the endangered Pyrenean population of bearded vulture. Our goals were to determine whether wild bearded vultures have experienced any Pb exposure events, and if so, to analyze exposure patterns.

2. Material and methods

2.1. Study species

The bearded vulture is a large bird of prey (5–7 kg) with a long lifespan that nests on rocky cliffs (Margalida and Bertran, 2000). Like other large birds, the bearded vulture has delayed sexual maturity, first breeding on average around 8 years of age (Antor et al., 2007), and it also has a very low rate of reproduction (Antor et al., 2007, 2009) although small mammals (e.g., wild rabbits (Oryctolagus cuniculus) et al., 2007) can also be important. Domestic species (n = 494 prey items) accounted for 72% of the diet (range 45–97.5%, n = 8).

2.2. Area and study population

Samples of bearded vulture populations from Spain (Navarra, Aragon and Catalonia; 96 breeding territories in 2008, Fig. 1) and France (29 breeding territories in 2008) were analyzed. The study has been carried out within the framework of conservation plans of the species in France and autonomous communities in Spain. In the study area, breeding bearded vultures feed principally on bone remains of wild and domestic ungulates (see Margalida et al., 2007, 2009) although small mammals (e.g., wild rabbits Oryctolagus cuniculus, European Hares Lepus europaeus) can also be important. Domestic species (n = 494 prey items) accounted for 72% of the diet (range 45–97.5%, n = 8).

Fig. 1. Regions considered in the study area of the bearded vulture in the Pyrenees (Spain and France).

2.3. Field procedures

One hundred and one blood samples from 87 wild bearded vultures were obtained from both fledglings in nests (n = 28) and individuals trapped during cannon netting (n = 73). The birds’ ages were determined using plumage characteristics: fledglings (n = 40); juveniles: individuals between one and three years old (n = 15); subadults: individuals between four and six years old (n = 20), and adults: individuals > 6 years old (n = 26). Adults were further categorized into non-territorial individuals (n = 6) and territorial individuals (n = 20) according to their breeding status and we took into account that in bearded vultures first-time pairing and territorial behavior were recorded when they were on average 6.5 years old (Antor et al., 2007). The samples were extracted from the brachial vein after the manual capture of the bird and were kept in commercial plastic tubes at 4 °C until their analysis. Each bearded vulture was identified by metal rings and wing tags and VHS or satellite radio transmitters. Eleven specimens were sampled twice and two further specimens were sampled three times. All samples from the same individuals were obtained at least 6 months apart and were considered as independent samples. Identification of gender was performed using blood in all but one individual by PCR amplification of the CHD-V gene, according to the technique of Ellegren (1996). Forty-eight individuals were males and 52 individuals, females.

Liver and bone tissue samples were obtained from 43 dead bearded vultures of all age classes, including fledglings (n = 12), juveniles (n = 3), subadults (n = 11) and adults (n = 17) including non-territorial (n = 3) and territorial (n = 8) individuals. All birds were necropsied by veterinary pathologists at official rehabilitation centers and the causes of mortality were described elsewhere (Margalida et al., 2008). Sex was determined at necropsy in 32 individuals and included 20 males and 12 females. The femur, humerus or tibia of each individual were extracted, which were mechanically cleaned with a scalpel to remove adhering muscle and tendon material. From each bone, a fragment approximately 1 cm long from the diaphysis was obtained, which was repeatedly washed in distilled water to remove any remains of bone marrow and traces from the cutting process. Liver tissue was not available for 13 individuals. Samples were stored frozen at −20 °C until their analysis.

2.4. Laboratory analysis

2.4.1. Lead analysis

Blood lead concentration was determined using graphite furnace atomic absorption spectrophotometry (AAS-CF) using a Perkin–Elmer model 3030 AAS equipped with a Perkin–Elmer HGA-600 graphite oven and a Perkin–Elmer AS-60 autosampler, following methodology described elsewhere (Pain et al., 1993). Blood Pb values are expressed as μg Pb/dl blood. Blank solutions were analyzed to verify Pb below detection limits. Sample detection limits ranged from 0.2 to 0.5 μg/dl. Recoveries of spiked samples ranged from 93% to 103% and averaged 96%. Aliquots of liver tissue (5 g) were weighed to determine wet weights, then they were freeze-dried and their dry weights were also recorded. Samples were digested in a solution of deionized water and 70% nitric acid at 1 ml solution per 0.1 g dry weight of the sample. Lead content in liver tissues was expressed as μg/g of liver on a dry weight basis. Recovery of Pb from spiked samples and standard solutions ranged from 67% to 113% and averaged 92%.

Lead concentration in bones was analyzed using flame-atomic absorption spectrophotometry using the Zeeman effect for background correction, as described by Amiard et al. (1987) on a Perkin–Elmer model 3030b spectrophotometer. Samples (0.5 g) were placed in plastic acid-washed test tubes and digested with 2.5 ml of 70% nitric acid for 12 h. Two and a half ml of 30% hydrogen peroxide were then added and the temperature was gradually increased to 160 °C in 1 h and held at that temperature for 4 h. The resulting digest was dissolved with hydrochloric acid and then diluted to a final volume of 10 ml with deionized water and analyzed. Pb concentrations in bone tissue were expressed as μg/g of bone on a dry weight basis. Detection limit for bone tissues was 0.32 μg/g dry weight per sample. Recovery of Pb from spiked samples and standard solutions ranged from 67% to 103.2% and averaged 92%.

Lead concentrations in wider environment far from lead emission sources were considered as background exposure; elevate exposure was considered when concentrations were found above these background concentrations: acute exposure was considered when exposure to Pb occurred in high Pb concentrations over a short period of time; Lastly, chronic exposure was considered when a sustained Pb exposure to lower Pb concentrations occurred (Pain, 1996).

In blood samples, a level < 20 μg/dl was considered to be indicative of “background” Pb exposure; individuals were considered to be “Pb exposed” when concentrations exceeded 20 μg/dl; and were “Pb poisoned”, thus showing clinical symptoms, when blood levels exceeded 40 μg/dl. These categories of Pb exposure
were based on published studies of blood concentrations associated with Pb poisoning in raptorial birds (Redig, 1991; Pain et al., 1993; Franson, 1996). In liver samples, <6 µg/g dw Pb was considered to be indicative of "background"; individuals were considered to be "Pb exposed" when concentrations exceeded 6 µg/g dw in liver; and were "Pb poisoned" when liver levels exceeded 20 µg/g dw according to the published studies (Pain et al., 1995; Franson, 1996; Clark and Scheuhammer, 2003). For bone concentrations, 20 µg/g was considered as the threshold level indicative of excessive exposure and absorption of Pb (Mateo et al., 2003; Pain et al., 2005).

2.4.2. Statistical analysis

Lead concentrations in blood, liver and bone samples were not normally distributed (Kolmogorov–Smirnov test, p < 0.01 for the three samples), so were log-transformed to fit a normal distribution. Undetected Pb blood values were considered as half of the limit of detection (LD, 0.1 µg/dl) for statistical purposes. Pb concentrations were compared among age classes and regions using an analysis of variance (ANOVA). When the ANOVA results proved to be statistically significant, a subsequent analysis was made employing the Scheffe test to identify differences between groups. In order to evaluate the differences between sexes and periods (hunting and non-hunting period), the data were analyzed using the t-test. For this, we considered the hunting period to run from August to February. Values of p = 0.05 were considered to be statistically significant.

3. Results

3.1. Acute exposure

The majority of samples had concentrations below 20 µg/dl (Fig. 2). Seven individuals (6.9%) had Pb concentrations > 10 µg/dl, but only two (1.9%) adult bearded vultures showed blood Pb levels above 40 µg/dl. Mean Pb concentration in blood (geometric mean 3.602 ± 0.767 [range LD–52.0] µg/ml) was far below the threshold indicative of acute exposure (Pain et al., 1993, 1995).

When age classes were considered (Table 1), blood Pb levels did differ significantly being higher in adult birds (F = 3.539, df = 3, 96, p = 0.02) than in fledglings (Scheffe test p = 0.03) but similar with respect to juveniles and subadults (Scheffe test, p = 0.993 and 0.611, respectively). Further, although blood Pb concentrations did not significantly differ between territorial and non-territorial individuals (t = −1.438, p = 0.163), differences between fledglings and adults were only significant (F = 3.874, df = 4.956, p = 0.01) in the case of territorial adults (Scheffe test, p = 0.01) but not in non-territorial adults (Scheffe test, p = 0.998).

Blood lead levels significantly differed between sampling regions (F = 6.726, df = 4, 96, p < 0.001, Table 2), being higher in the eastern range of distribution (Catalonia) than in the central range of distribution (Aragon) (Scheffe test, p < 0.001). However, the age and region variables are not independent (χ² = 31.06, df = 9, p = 0.0003) with adult samples more represented in Catalonia (66.7%, n = 12) than in Aragón (11.4%, n = 79) and France (33.3%, n = 6).

Blood Pb concentrations between the sexes did not differ significantly (t = 0.405, p = 0.687, Table 3). Blood lead levels were not significantly different between hunting seasons (geometric mean 3.899 ± 0.745, range LD–47.0, n = 54) and non-hunting season (geometric mean 3.288 ± 0.763, range LD–52.0, n = 47, t = 0.324, p = 0.747).

The mean liver Pb concentration (geometric mean 0.942 ± 0.921 µg/g dw [range 0.15–22.0]) was also far below the threshold indicative of recent exposure. However, one adult individual showed elevated liver Pb concentrations (9.07 µg/g dw) indicating recent Pb exposure and another adult individual showed liver Pb concentrations (22.0 µg/g dw) indicative of Pb poisoning (Pain et al., 1995; Franson, 1996; Clark and Scheuhammer, 2003). Liver lead concentrations significantly differed between regions (F = 3.548, df = 3, 26, p = 0.028, Table 3), higher in France than in Aragón and Catalonia. Liver lead levels increased with age, but differences between age classes were not significant (F = 0.874, df = 3, 26, p = 0.467, Table 1). However, bone Pb levels were higher in territorial adults and subadults than in other age classes (Table 1). Although liver lead concentrations were higher during the hunting season (geometric mean = 1.181 ± 0.687, n = 8) than outside of the hunting season (geometric mean = 0.868 ± 4.585, n = 22), differences were not significant.

![Fig. 2. Distribution of blood Pb concentrations (µg/dl) in the Pyrenean bearded vulture population.](image-url)

<table>
<thead>
<tr>
<th>Table 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood (µg/dl), liver and bone (µg/g) lead levels (geometric mean ± SD and range) in different ages in the Pyrenean bearded vulture population.</td>
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<tr>
<td></td>
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<tr>
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<tr>
<td></td>
</tr>
<tr>
<td>Territorial adult</td>
</tr>
<tr>
<td>Non-territorial adult</td>
</tr>
<tr>
<td>Adult</td>
</tr>
<tr>
<td>Subadult</td>
</tr>
<tr>
<td>Juvenile</td>
</tr>
</tbody>
</table>

ND: not detected.
Table 2
Blood (\(\mu g/dl\)), liver and bone (\(\mu g/g\)) lead levels (geometric mean \(\pm\) SD and range) in the Pyrenean bearded vulture population according to the geographical regions.

<table>
<thead>
<tr>
<th>Region</th>
<th>Blood</th>
<th>Liver</th>
<th>Bone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n)</td>
<td>Mean (\pm) SD (range)</td>
<td>(n)</td>
</tr>
<tr>
<td>Spain</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aragón</td>
<td>79</td>
<td>3.132 (\pm) 2.894 (ND–18.9)</td>
<td>19</td>
</tr>
<tr>
<td>Cataluña</td>
<td>12</td>
<td>8.823 (\pm) 14.476 (2.0–52.0)</td>
<td>7</td>
</tr>
<tr>
<td>Navarra</td>
<td>4</td>
<td>2.449 (\pm) 0.5774 (2.0–3.0)</td>
<td>1</td>
</tr>
<tr>
<td>Francia</td>
<td>5</td>
<td>5.080 (\pm) 19.474 (ND–47.0)</td>
<td>3</td>
</tr>
<tr>
<td>Andorra</td>
<td>1</td>
<td>5.0</td>
<td></td>
</tr>
</tbody>
</table>

Table 3
Differences between sexes in blood (\(\mu g/dl\)), liver and bone (\(\mu g/g\)) lead levels (geometric mean \(\pm\) SD and range) in the Pyrenean bearded vulture population.

<table>
<thead>
<tr>
<th>Region</th>
<th>Blood</th>
<th>Liver</th>
<th>Bone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n)</td>
<td>Mean (\pm) SD (range)</td>
<td>(n)</td>
</tr>
<tr>
<td>Male</td>
<td>48</td>
<td>3.335 (\pm) 7.017 (ND–47.0)</td>
<td>14</td>
</tr>
<tr>
<td>Female</td>
<td>52</td>
<td>3.850 (\pm) 8.158 (ND–52.0)</td>
<td>6</td>
</tr>
<tr>
<td>Unknown</td>
<td>1</td>
<td>4.5</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 3. Distribution of bone lead concentrations (\(\mu g/g\) dw) in the Pyrenean bearded vulture population.

\((t = -0.279, p = 0.782)\). However, when obvious outliers to the distribution (liver lead concentration from two individuals) were excluded, differences were significant \((t = 3.547, p = 0.002)\). Pb concentrations in liver did not significantly differ between the sexes \((t = -1.801, p = 0.09, Table 2)\).

3.2. Chronic exposure

Mean bone Pb concentrations (geometric mean 2.457 \(\pm\) 6.032 \(\mu g/g\) dw, Fig. 3) were far below the threshold indicative of chronic exposure \((Pain et al., 2005; Mateo et al., 2003)\), but one (2.3%) of the studied individuals \((n = 43)\) showed bone Pb concentration to be indicative of chronic poisoning \((>20 \mu g/g, Gangoso et al., 2009)\). Distribution of bone Pb concentrations ranged from 0.32 to 40.5 \(\mu g/g\) dw.

Bone Pb concentrations did not significantly differ between regions \((F = 2.389, df = 2, 40, p = 0.104, Table 1)\) or sexes \((t = -1.545, p = 0.132, Table 3)\). Bone lead concentrations were slightly higher during the hunting season (geometric mean = 2.782 \(\pm\) 10.614, \(n = 13\)) than outside of the hunting season (geometric mean = 2.328 \(\pm\) 2.037, \(n = 30, n = 30\)) but differences were not significant \((t = 1.250, p = 0.218)\). When age classes were considered, bone lead levels differed \((F = 3.582, df = 3, 39, p = 0.022, Table 1)\), significantly different between fledglings and subadults \((\text{Scheffé}, p = 0.04)\). Further, when non-territorial \((n = 3)\) and territorial \((n = 8)\) adult individuals were considered, differences were maximal and significant between fledglings and territorial adults \((F = 2.954, df = 4, 32, p = 0.04)\).

4. Discussion

The present study, a survey of simultaneous recent, medium-term and long-term exposure of lead in a raptor species, is carried out for the first time in Europe. Acute Pb poisoning has been recognized as one of the most important causes of mortality of raptors \((Pain et al., 1995; Mateo et al., 2003; Fisher et al., 2006; Snyder, 2008)\). As obligate scavengers, vultures are potentially susceptible to the ingestion of lead shot, as they can be exposed to high lead concentrations even when feeding on body parts far from where the bullet was embedded \((Fisher et al., 2006; Snyder, 2008; Hall et al., 2007; Hunt et al., 2009)\). Available evidence indicates that Pb poisoning in adult, free-living raptors is almost exclusively due to dietary exposure to fragments of metallic Pb from Pb ammunition used in hunting \((Cade, 2007; Green et al., 2008)\). In addition, there are no sources of lead from active industries or mining in the range of distribution of the Pyrenean population of the bearded vulture, hence lead exposure in bearded vultures showing elevated tissue Pb concentrations is not likely to be related to environmental pollution. However, further comparison of Pb isotope ratios need to be conducted to identify Pb exposure routes in vultures showing background concentrations \((Cade, 2007; Pain et al., 2007)\).

Blood Pb level is a good indicator of acute exposure, whereas bone lead levels represent repeated, chronic exposure, as bones are long-term repositories of lead \((Pain et al., 1993; Franson, 1996; Clark and Scheuhammer, 2003; Gangoso et al., 2009)\). Organotropism may therefore be useful to distinguish acute and chronic exposure \((Ancora et al., 2008)\). Pb levels in liver and kidney may be indicative of medium-term exposure, as they act as the primary organs in which this metal accumulates \((Pain et al., 1996)\). However, few studies try to characterize recent, medium-term and chronic exposure and accumulation of lead simultaneously.
Birds acutely exposed to lead may die with elevated soft tissue concentrations, but before much lead has been deposited in bone (Patte et al., 1981; Pain et al., 2005). Conversely, birds may accumulate a certain amount of lead in bone over time from non-lethal acute exposure, with no signs of toxicity (Pain et al., 2005).

In the current study, 93% (n = 94) of bearded vulture blood samples showed Pb concentrations far below the threshold indicative of recent exposure and therefore were considered as exposed to “background” concentrations and probably they have nothing to do with hunting activities. However, five bearded vulture blood samples showed Pb concentrations above 10 μg/dl and two individuals had a Pb blood level above the threshold of 40 μg/dl that we used as an indicator for Pb poisoning. An immature individual showing a blood Pb level of 45.0 μg/dl was found emaciated, but clinical examination and supporting tests revealed no abnormalities other than severe dehydration, which is considered to be the cause of increased blood Pb levels (Lumeij, 1985; Franson, 1996). At low or medium concentrations, one single incidence of lead detection does not allow the determination of the degree of exposure as it is impossible to determine the time elapsed between exposure and sampling (Franson, 1996; Hall et al., 2007; Snyder, 2008; Green et al., 2008). An adult individual showing 52.0 μg/dl was found dead 22 months after the sampling showed high liver and bone Pb concentrations (9.07 and 40.5 μg/g dw, respectively) indicative of Pb poisoning. This finding suggests that thresholds established for other birds of prey (Redig, 1991; Pain et al., 1993, 1995, 2005; Franson, 1996; Clark and Scheuhammer, 2003; Mateo et al., 2003) are also suitable for the bearded vulture.

Liver and bone Pb concentrations found in bearded vultures supported results of blood lead levels. Most of the individuals included in this study showed Pb concentrations above the thresholds indicative of recent or chronic exposure. However, an adult bird showing elevated liver Pb concentrations and another adult bird showing a liver concentration indicative of Pb poisoning (Pain et al., 1995; Franson, 1996; Clark and Scheuhammer, 2003) suggest that the Pyrenean population is not free from poisoning risk. Although Pb at concentrations below those known to cause acute death is known to cause severe health effects, including the increase in susceptibility to diseases and accidents (Wayland et al., 2003; Clark and Scheuhammer, 2003; Pain et al., 2007), Pb is unlikely to have been a contributory factor in the mortality of the remainder of the studied bearded vultures. Causes of mortality of individuals sampled in this study included intentional and unintentional poisoning by other toxic agents, principally organophosphate and carbamate insecticides, shooting, electrocution and collision with power wires (Margalida et al., 2008), and there was no evidence to suggest that sublethal effects of Pb at background concentrations found bearded vultures predisposed to injury or disease. Nevertheless, significantly higher blood Pb levels are found in adult individuals than in juveniles and fledglings (Table 1). These differences may be explained by different feeding habits and strategies and potential Pb shot ingestion (Wayland et al., 2003; Gangoso et al., 2009) between juveniles and fledglings and other age classes, as these two categories of individuals are more dependent on predictable food sources (supplementary feeding sites) or different food sources (see Oro et al., 2008; Margalida et al., 2009), which may reduce the incidence of Pb exposure (Cade, 2007; Snyder, 2008). In this sense, bone lead concentrations were only significantly lower in fledglings in relation to other age classes, but our results did not show an age-related bioaccumulation effect in the population. This may be an important issue for the population dynamics because bearded vultures are long lived, have low annual reproductive potential with an older age of first breeding such that population levels are linked to the survival of breeding adults (Oro et al., 2008) as observed in other large raptors (Wayland et al., 2003; Whitfield et al., 2004; Hernández and Margalida, 2008; Ortega et al., 2009) and accumulation of lead at subclinical levels could be the cause of mortality and morbidity (Wayland et al., 2003; Pain et al., 2007; Gangoso et al., 2009). From a conservation point of view, the provision of supplementary food at artificial feeding sites is proved to be a useful tool for preventing indirect poisoning, thus increasing the survival (Oro et al., 2008). Our results suggest that this management technique could help to reduce lead exposure among individuals in those regions with more lead poisoning risk.

Although there may be sex differences in the probability of exposure or poisoning by lead (Pain et al., 1993; Fisher et al., 2006), in our study blood, liver and bone Pb concentrations between sexes did not differ significantly. This may be the result of similar feeding habits and strategies between sexes (Margalida and Bertran, 2000).

Blood, liver and bone lead levels found in the bearded vultures were slightly higher during the hunting season than outside of the hunting season. We consider the obvious source of Pb exposure for the individuals showing elevated blood, liver and bone levels of Pb to be the ingestion of lead gunshot in their prey. Radiological examination of one of these individuals showed no shot pellets or bullet fragments in the digestive tract or embedded in its flesh, confirming that the source of blood Pb levels was the ingestion of lead gunshot in their prey. This was in accordance with other studies in which raptors with high Pb levels tended to be found during or soon after the end of the hunting season (Pain et al., 1995, 2005, 2007; Mateo et al., 2003; Green et al., 2008; Gangoso et al., 2009), whereas those with background Pb levels were found more often long after the hunting season had ended (Wayland et al., 2003).

5. Conclusions

Vultures may be at particularly high risk for ingestion of fragments of Pb ammunition embedded in dead game animals (Clark and Scheuhammer, 2003; Green et al., 2008; Hunt et al., 2009). Our results indicate that, like the California condor and other raptor species, lead poisoning represents a threat to the Pyrenean bearded vulture population. This species is not free from the risk of lead poisoning, principally the breeding population, given the age of individuals showing elevated blood, liver and bone lead levels. Lead presents an unnecessary threat to adult birds, since they more frequently forage and search for prey, including game birds and mammals (Margalida et al., 2008). The establishment of predictable food sources as feeding stations has been considered as an important tool for the conservation of species in Spain, since they help to reduce non-adult mortality (see Heredia, 1991; Oro et al., 2008). However, their elimination or reduction may increase the potential risk of Pb poisoning as non-adult bearded vultures may be forced to search for less predictable food sources that may include prey containing shot pellets or bullet fragments in their flesh (Clark and Scheuhammer, 2003; Pain et al., 2005; Cade, 2007; Gangoso et al., 2009). The only way to remove this risk is to ban all hunting with lead within the range of distribution of the bearded vulture since in countries where lead shot has been restricted or banned, the prevalence of lead exposure has declined since the ban (Ancora et al., 2008; Cade, 2007). While this measure is implemented, carcasses and offal of ungulates killed by hunters should be retrieved to avoid any preventable additional mortality in this small endangered population, principally in the Pyrenean eastern subpopulation.

Regular monitoring based on lead analyses from regular field
sampling could also be important to reveal recent exposure to lead as well as to provide information on the cause of death of individuals and to gain information on the long-term effects of lead (Pain et al., 2007; Gangothi et al., 2009). Acute or chronic lead poisoning should also be considered in differential diagnosis in any diseased or injured wild bearded vulture, especially in the case of subadult and adult individuals. The potential risk of Pb poisoning should also be a priority factor to be evaluated before bearded vulture reintroduction or reinforcement programs are implemented.

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References