# Short Communications

## Lead Exposure in the Critically Endangered Bearded Vulture (*Gypaetus barbatus*) Population in Southern Africa

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Abstract.—Lead poisoning is an important threat to some raptor species, and the primary source of lead is through the ingestion of carcasses that have been shot with lead, which makes scavengers such as vultures particularly vulnerable. We examined the concentrations of lead in blood and bone tissue samples collected throughout the range of the Bearded Vulture (*Gypaetus barbatus*) in southern Africa. This population is regionally critically endangered and it is not known to what extent lead poisoning may be a threat. Blood lead levels ($0.62 \pm 0.81$ µg dL$^{-1}$) from six live birds tested in 2017 indicated background levels of exposure to lead. Similarly, five live birds tested in 2009 using a less sensitive method all had blood lead levels <3 µg dL$^{-1}$. Bone lead levels ($11.79 \pm 8.34$ µg g$^{-1}$) from eight birds that had died indicated lead exposure and accumulation over time, suggesting that lead may have contributed to their deaths. These levels of lead may be detrimental to the survival and fecundity of this small and declining population. Recommendations to address this threat include banning hunting and culling with lead ammunition, which is the most likely source of this pollutant. Such actions may reduce the population's susceptibility to other threats, which may be compounded by high lead levels, and help ensure the success of planned reintroduction programs.

Key Words: *Bearded Vulture*; Gypaetus barbatus; *Africa*; *ammunition*; *lead poisoning*; *population*; *threat*.

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## EXPOSICIÓN AL PLOMO EN POBLACIONES EN PELIGRO CRÍTICO DE *GYPAETUS BARBATUS* EN EL SUR DE ÁFRICA

Resumen.—El envenenamiento por plomo es una amenaza importante para algunas especies de rapaces. La fuente principal de plomo es la ingesta de cadáveres que han sido abatidos con plomo, lo que hace que los carroñeros como los buitres sean particularmente vulnerables. Examinamos las concentraciones de plomo en muestras de sangre y tejido óseo recolectadas a lo largo del área de distribución de *Gypaetus barbatus* en el sur de África. Esta población está en peligro crítico a nivel regional y no se sabe en qué medida el envenenamiento por plomo puede ser una amenaza. Los niveles de plomo en sangre ($0.62 \pm 0.81$ µg dL$^{-1}$) en seis aves vivas evaluadas en 2017 indicaron niveles de fondo de exposición al plomo. De modo similar, cinco aves vivas evaluadas en 2009 usando un método menos sensible mostraron en todas ellas niveles de plomo en sangre <3 µg dL$^{-1}$. Los niveles de plomo en tejido óseo ($11.79 \pm 8.34$ µg g$^{-1}$) de ocho aves muertas indicaron exposición al plomo y acumulación a lo largo del tiempo, sugiriendo que el plomo puede haber contribuido a sus muertes. Estos niveles de plomo pueden ser perjudiciales para la supervivencia y la fecundidad de esta pequeña población en disminución. Las recomendaciones para abordar esta amenaza incluyen la prohibición de caza y sacrificio con munición de plomo, la que representa la fuente más probable de este contaminante. Tales acciones pueden reducir la susceptibilidad

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de la población a otras amenazas, que pueden verse agravadas por los altos niveles de plomo, y ayudar a asegurar el éxito de los programas de reintroducción planificados.

[Traducción del equipo editorial]

Lead (Pb) is a highly toxic heavy metal and lead poisoning manifests itself as a cumulative, multi-systemic condition affecting all body systems (Locke and Thomas 1996, Pain 1996, Redig and Cruz-Martínez 2009). Lead poisoning is the most frequent form of heavy metal poisoning in birds of prey and is one of the most important causes of mortality of raptors worldwide (Franson 1996, Mautino 1997, Pain et al. 2005, Fisher et al. 2006). Lead poisoning has been implicated in the decline of a number of critically endangered raptors (Kramer and Redig 1997, Kenntner et al. 2001, Clark and Scheuhammer 2003, Pain et al. 2005) including the California Condor (*Gymnogyps californianus*; Green et al. 2008) and a number of Old World vultures such as the Griffon Vulture (*Gyps fulvus*), Pyrenean Bearded Vulture (*Gypaetus barbatus*), Cinereous Vulture (*Aegypius monachus*) and Egyptian Vulture (*Neophron percnopterus*; Hernández and Margalida 2009, Nam and Lee 2009, Rodriguez-Ramos et al. 2009, Kelly et al. 2011, Lambertucci et al. 2011, Stringfield 2012).

Consumption of prey containing lead shot or bullet fragments is generally perceived as the primary source of lead poisoning in raptors (Kendall et al. 1996, Mateo et al. 1999, Clark and Scheuhammer 2003, Fisher et al. 2006), and for a number of species this has been confirmed through isotopic analysis (Finkelstein et al. 2014, Madry et al. 2015). The high velocities of modern firearms cause lead-based bullets to fragment widely along the wound tract (Stroud and Hunt 2009). The level of fragmentation makes scavengers, such as vultures, highly susceptible to lead poisoning from carcasses or gut piles left in the field (Clark and Scheuhammer 2003, Green et al. 2008, Hunt et al. 2009). Vultures dissolve and absorb lead soon after ingestion due to their low stomach pH, often resulting in acute death (García-Fernández et al. 2005, Fisher et al. 2006). Sublethal or chronic exposure (prolonged exposure at lower concentrations) is also of concern because it can make birds less fit and predispose them to other causes of death (Mateo et al. 2003, Pain et al. 2007, Berny et al. 2015) or may affect reproductive success (Buerger et al. 1986, Gil-Sánchez et al. 2018), behavior (Scheuhammer 1987), or physiology (Burger 1995, Fair and Ricklefs 2002, Naidoo et al. 2012).

Blood lead levels are a good indicator of recent exposure and the degree of lead poisoning over short periods of time (Tirelli et al. 1996), because the half-life of lead in blood is 14 d (Frey et al. 2003, Pain et al. 2009). Bone lead levels represent repeated exposure and absorption of lead, because bones are long-term repositories of lead (Pain et al. 1993, Franson 1996, Clark and Scheuhammer 2003, Gangoso et al. 2009). Long-lived species, such as vultures,

are particularly susceptible to the bioaccumulation of lead in bone tissue (Gangoso et al. 2009, Behmke et al. 2015).

Vulture populations across Africa have undergone massive declines, with seven species declining at a rate of >80% over three or more generations. The declines have been attributed mainly to poisoning and trade in traditional medicines (Ogada et al. 2016). Similarly in southern Africa, the Bearded Vulture population has declined by >30% during recent decades and has been listed as regionally critically endangered with only approximately 100 pairs remaining (Krüger et al. 2014a, Krüger 2015). The influence of lead poisoning on the decline of the vulture populations in Africa has been relatively poorly explored. However, recent research has suggested that it may be a more significant issue than previously appreciated (Naidoo et al. 2017, Garbett et al. 2018). We examined the blood lead levels of captured individuals and bone lead levels of Bearded Vultures found dead throughout their range in southern Africa to determine the level of exposure, and to ascertain whether lead poisoning may pose an important threat to this small and isolated population.

METHODS

**Study Area and Data Collection**. Lead levels of Bearded Vultures were determined from blood samples (11) and bone samples (8) collected across the species' southern African range in the Maloti Mountains of Lesotho ($n = 1$) and in the Drakensberg Mountains of the KwaZulu-Natal ($n = 11$), Free State ($n = 5$) and Eastern Cape ($n = 2$) provinces of South Africa (Fig. 1).

Blood was collected from individuals that were captured at supplementary feeding sites between 2009 and 2012 when birds were fitted with satellite transmitters ($n = 10$), and marked as a nestling in the nest ($n = 1$). Blood was taken from the brachial vein using a sterile hypodermic needle and syringe, and samples were immediately transferred into EDTA coated tubes. Five of the blood samples, collected in 2009, were submitted to a commercial laboratory (Ampath Pty. Ltd., Pretoria, Gauteng, South Africa) and whole blood lead concentrations were determined using graphite furnace atomic absorption spectrometry (GFAAS) following a preparation step with 0.1% Triton X-100. Results from these five samples were reported previously (Naidoo et al. 2017). The remaining six samples were analyzed by V&M Analytical Toxicology Laboratory Services Pty. Ltd. (George, Western Cape, South Africa), using the Inductively Coupled Plasma Mass Spectrometer (ICP-MS) method.
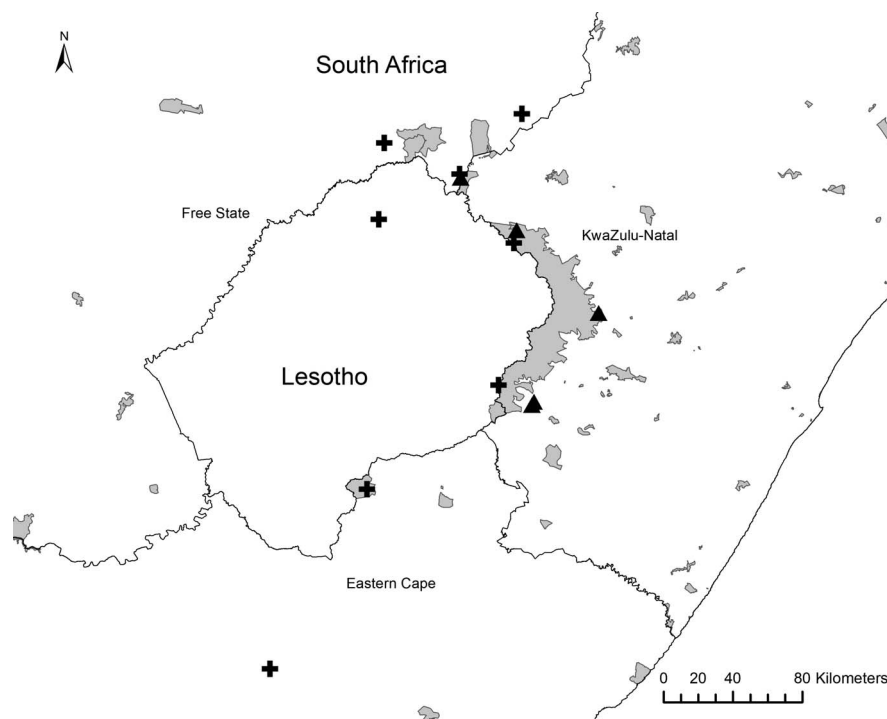
Figure 1. Location of blood collection sites (▲) and bone collection sites (✚) within the Bearded Vultures' range in the Maloti-Drakensberg Mountains of southern Africa.

Bone samples were collected from seven individuals that were tracked with GPS tags (see Krüger et al. 2014b for more details) and found dead during the study period (2009–2017), and one non-tagged individual that was opportunistically found dead. Four of these individuals had been tested for blood lead levels when they were captured in 2009 ($n = 2$) and 2012 ($n = 2$). The bone lead concentrations were determined by (1) Biochemical and Scientific Consultants Pty. Ltd. (Hilton, KwaZulu-Natal, South Africa), using the GFAAS method ($n = 6$) and the ICP-MS method ($n = 1$), and (2) V&M Analytical Toxicology Laboratory Services Pty. Ltd. using the ICP-MS method ($n = 1$). The quantitative determination of lead in blood/bone by the ICP-MS method is a more modern technique with wider application scope and was used on the more recent samples because it is a more accurate and sensitive method.

**Lead Concentration.** In raptors and vultures, blood lead levels $<20$ µg dL$^{-1}$ indicate background levels of exposure, levels of 20–40 µg dL$^{-1}$ indicate exposure, and levels $>40$ µg dL$^{-1}$ indicate lead poisoning (Redig 1991, Pain et al. 1993, Franson 1996, Kelly and Johnson 2011). For bone, lead levels $<10$ µg g$^{-1}$ indicate low level background exposure, levels of 10–20 µg g$^{-1}$ indicate abnormal exposure, and levels $>20$ µg g$^{-1}$ indicate pathological

exposure and absorption of lead (Mateo et al. 2003, Pain et al. 2005). Descriptive values are presented as mean ± standard deviation.

**Ethics Statement.** Vulture capture and blood collection procedures were approved by the Animal Ethics Committee of the Science Faculty of the University of Cape Town (reference: 2011\V14\SK), South African National Parks and Ezemvelo KwaZulu-Natal Wildlife (Research Project Registration number W/2057/01). The capture and handling of vultures was executed under the Endangered Wildlife Trust's Threatened or Protected Species registration certificate granted by the Gauteng Provincial Department of Agriculture, Conservation and Environment, South Africa (permit: 07046).

RESULTS AND DISCUSSION

A total of 19 samples from 15 individuals were tested for lead levels (Table 1). The average bone lead concentration in our study was $11.79 \pm 8.34$ µg g$^{-1}$ (0.50–20.92 µg g$^{-1}$, $n = 8$), which suggests that the individuals were exposed to lead. Two non-adults had concentrations suggestive of pathological exposure and absorption of lead (19.96 and 20.92 µg g$^{-1}$), which may well have been the cause of the

Table 1.   Bone ($\mu g\ g^{-1}$) and blood ($\mu g\ dL^{-1}$) lead concentrations in the southern African Bearded Vulture population obtained from birds across the Maloti-Drakensberg Mountain region. EC is Eastern Cape province, KZN is KwaZulu-Natal province, FS is Free State province, and LES is Lesotho. Letters in parentheses after Pb levels indicate whether the individual was an adult (A) or non-adult (N) at the time the sample was taken.

| INDIVIDUAL | DATE | SEX | BONE PB | BLOOD PB | DATE AND CAUSE OF DEATH | LOCATION |
|---|---|---|---|---|---|---|
| G27303 | November 2008 | Female | 15.50 (N) | - | April 2011, not confirmed[b] | EC |
| G27305 | August 2009 | Female | 5.20 (A) | <3[a] (N) | May 2015, not confirmed[b] | KZN (blood) LES (bone) |
| G27306 | August 2009 | Male | 19.96 (N) | <3[a] (N) | May 2013, not confirmed[b] | KZN (blood) FS (bone) |
| G27307 | August 2009 | Male | - | <3[a] (N) | n/a | KZN |
| G27308 | August 2009 | Male | - | <3[a] (N) | August 2009, power line collision | KZN |
| G27309 | August 2009 | Female | - | <3[a] (N) | n/a | KZN |
| G27313 | August 2010 | Male | - | 0.01 (N) | n/a | KZN |
| G27314 | August 2010 | Female | 14.90 (A) | - | February 2013, not confirmed[b] | KZN |
| G27315 | July 2010 | Female | 20.92 (N) | - | November 2012, not confirmed[b] | FS |
| G27376 | September 2011 | Female | - | 0.55 (A) | n/a | KZN |
| G27377 | August 2012 | Male | - | 0.96 (A) | n/a | KZN |
| G27381 | August 2012 | Male | 16.60 (A) | 0.11 (A) | February 2014, carbamate poison | FS |
| G27383 | August 2012 | Male | 0.71 (A) | 0.01 (N) | n/a | KZN |
| G27384 | September 2012 | Male | - | 2.08 (N) | n/a | KZN |
| ONR[c] | September 2012 | Unknown | 0.50 (A) | - | September 2012, unknown | EC |

[a] Results reported in Naidoo et al 2017.
[b] In all cases where the cause of death was not confirmed, birds showed signs of poisoning (lying face down with wings outstretched) in the field.
[c] Opportunistically collected after death, thus no ring, at Ongeluksnek Nature Reserve.

birds' deaths, as levels $>20\ \mu g\ g^{-1}$ indicate chronic poisoning (Gangoso et al. 2009) and concentrations $>20$–$30\ \mu g\ g^{-1}$ were found in birds that had died of lead poisoning (Pain et al. 2007).

The average blood lead concentration using the more sensitive analysis (ICP-MS) was $0.62 \pm 0.81\ \mu g\ dL^{-1}$ (0.00–$2.08\ \mu g\ dL^{-1}$, $n = 6$); however, concentrations measured using the less sensitive GFAAS analysis were all classifed as $<3\ \mu g\ dL^{-1}$, the limit of quantification for this method ($n = 5$) and therefore not included in the graphical representation of our results. All our blood samples had lead levels far below those indicative of lead exposure (20–40 $\mu g\ dL^{-1}$), suggesting only background levels of exposure to lead.

For two birds we had blood samples collected when they were alive and bone samples collected following their deaths 2 yr and 4 yr later. In both cases their blood levels were relatively low ($<3.0\ \mu g\ dL^{-1}$), but their bone lead levels showed higher levels of exposure (19.96 $\mu g\ g^{-1}$ and 16.60 $\mu g\ dL^{-1}$ respectively). These results are suggestive of a temporal change in exposure and an accumulation of lead in the bone tissue over time. Although samples sizes are small, it would appear that both males and females of all ages may be at risk (Table 1). Our results suggest that while the southern African Bearded Vulture population does not show signs of recent or short-term exposure to lead, it does show exposure to lead over the long term.

Based on average blood lead concentrations, the lead exposure of the southern African Bearded Vulture population ($<3\ \mu g\ dL^{-1}$) is similar to that of Bearded Vultures in the Pyrenees (3.6 $\mu g\ dL^{-1}$; Hernández and Margalida 2009). It also appears somewhat lower than that of Cape Vultures (*Gyps coprotheres*) in southern Africa (12.5 $\mu g\ dL^{-1}$; Naidoo et al. 2017), African White-backed Vultures (*Gyps africanus*) in South Africa (7.81 ug $dL^{-1}$; Van Wyk et al. 2001 and 15.5 $\mu g\ dL^{-1}$; Naidoo et al. 2017) and Botswana (10.7 $\mu g\ dL^{-1}$; Garbett et al. 2018), but well below that found in Griffon Vultures exposed to lead bullets in Spain (43.07 $\pm$ 3.96 $\mu g\ dL^{-1}$; García-Fernández et al. 2005; Fig. 2).

However, bone lead concentrations in this study (11.8 $\mu g\ g^{-1}$), were higher than those found in Pyrenean Bearded Vultures (2.5 $\mu g\ g^{-1}$; Hernández and Margalida 2009) and Egyptian Vultures on the Iberian Peninsula (6.2 $\mu g\ g^{-1}$) and Canary Islands (7.4 $\mu g\ g^{-1}$; Gangoso et al. 2009) but were lower than those of White-backed Vultures in South Africa (27.1 $\mu g\ g^{-1}$; van Wyk et al. 2001; Fig. 3).

Lead toxicity in avian species manifests itself as a cumulative, multi-systemic syndrome affecting the liver, kidney, heart, gastrointestinal, hematopoietic, reproductive, and nervous systems (Locke and Thomas 1996, Redig and Cruz-Martínez 2009). The ultimate consequences of poisoning by lead can be readily underestimated, given that the sublethal effects are difficult to detect and may only
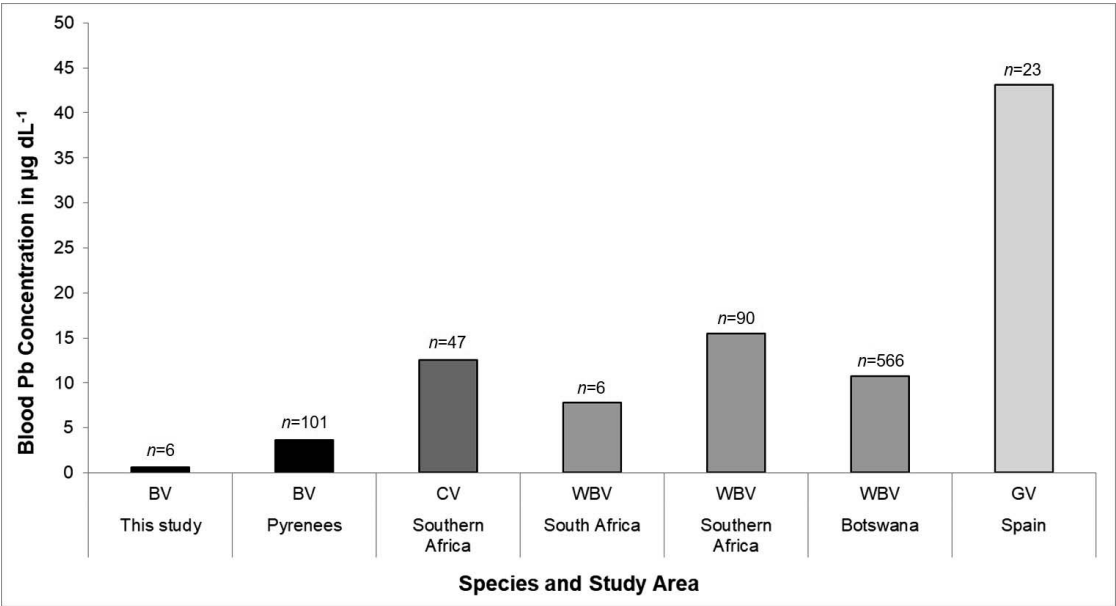
Figure 2. A comparison of the average blood lead (Pb) concentration (measured in µg dL$^{-1}$) of the southern African Bearded Vulture population with those of the Bearded Vulture (BV; black bars) in the Pyrenees (Hernandez and Margalida 2009), the Cape Vulture (CV; dark gray bars) in southern Africa (Naidoo et al 2017), the African White-backed Vulture (WBV; medium gray bars) in South Africa (van Wyk et al. 2001), southern Africa (Naidoo et al. 2017) and Botswana (Garbett et al. 2018), and the Griffon Vulture (GV; light gray bars) in Spain (García-Fernández et al. 2005).
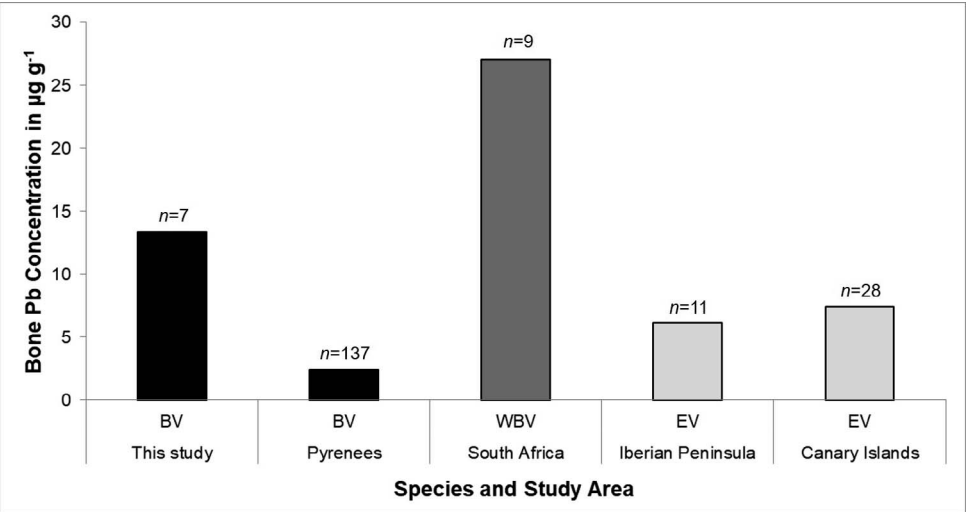
Figure 3. A comparison of the average bone lead (Pb) concentration (measured in µg g$^{-1}$) of the southern African Bearded Vulture (BV; black bars) population with those of the Bearded Vulture (BV) in the Pyrenees (Hernández and Margalida 2009), the African White-backed Vulture (WBV; dark gray bars) in South Africa (van Wyk et al. 2001), and the Egyptian Vulture (EV; light gray bars) on the Iberian Peninsula and Canary Islands (Gangoso et al. 2009).

show over a very long time period and in combination with other factors (Franson et al. 1983, Mateo et al. 1997, Smits et al. 2007). For example, the accumulation of lead in bones from lead ammunition affects the degree of bone mineralization in Egyptian Vultures, which could mean an increase in bone fragility (Fleming et al. 2000, Whitehead and Fleming 2000). The accumulation of lead over time is an additional threat to the species because it can affect the fitness of a population, including reproduction (Gil-Sánchez et al. 2018), thus affecting the long-term survival of long-lived species. The effects of the cumulative exposure to lead on this population are difficult to assess with the low sample sizes in this study. However, lead exposure may render individuals more susceptible to other agents of mortality, such as poisoning and collisions with power lines or cables (Berny et al. 2015), which may in turn affect population dynamics.

Lead is a highly toxic heavy metal, which can be released into the environment mainly by industrial, mining, and hunting activities (Fisher et al. 2006). South Africa is known to have high soil and air lead concentrations linked to mining activities (Herselman 2007, Mathee 2014). Although there are no major industries within the species' distribution range in southern Africa, some localized mining does occur. However, Naidoo et al. (2017) found no relationship between blood lead levels in vultures in southern Africa and vicinity to mines or soil lead concentrations, and three soil samples collected from within our study area had background lead levels ($<10$ μg $g^{-1}$, S. Krüger unpubl. data). Naidoo et al. (2017) concluded that vultures in southern Africa are exposed to another source of lead, with lead bullets being the most likely source. In Botswana, elevated blood lead levels in White-backed Vultures were closely associated with hunting seasons and hunting areas, providing strong support for the idea that spent lead ammunition is the major source of lead exposure in southern African vultures (Garbett et al. 2018).

Lead poisoning, resulting from feeding on carcasses shot with lead ammunition, is one of the main threats to the species in the Pyrenees (Hernández and Margalida 2009, Berny et al. 2015) and vultures elsewhere (Gongoso et al. 2009, Mateo 2009, Nam and Lee 2009, Rodriguez-Ramos et al. 2009, Kelly et al. 2011, Lambertucci et al. 2011, Stringfield 2012). Although hunting activities within the species' southern African range are limited, there are numerous supplementary feeding sites where animals are placed after they have died or been shot with lead ammunition. Lead presents an unnecessary additional threat to the species; therefore, we recommend the ban of hunting or culling with lead within the species' distribution range and the replacement of lead-based ammunition with nontoxic alternatives. We also advocate an education program aimed at those managing feeding sites, to ensure that animals killed using lead ammunition are properly processed or not placed out at all.

Our results provide a preliminary indication that lead poisoning is an additional threat to the southern African Bearded Vulture population that may predispose individuals to other threats (Berny et al. 2015). Further studies are required to ascertain whether lead poses a significant threat to populations of this threatened species in the long term by affecting reproductive success or survival (Gil-Sanchez et al. 2018). The potential risk of lead poisoning must also be considered in future reintroduction programs, which are actively being considered for the region (Brink 2016).

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