Blood and bone lead levels in South Africa's *Gyps* vultures: Risk to nest-bound chicks and comparison with other avian taxa

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Highlights

• Lead can impair chick development and enhance vulnerability to anthropogenic threats.

• A significant proportion of evaluated *Gyps* vultures (including chicks) were exposed to lead.

• In comparison non-scavenging birds did not display elevated lead concentrations.

• Nest-bound chicks likely received metallic lead fragments from their parents during feeding.

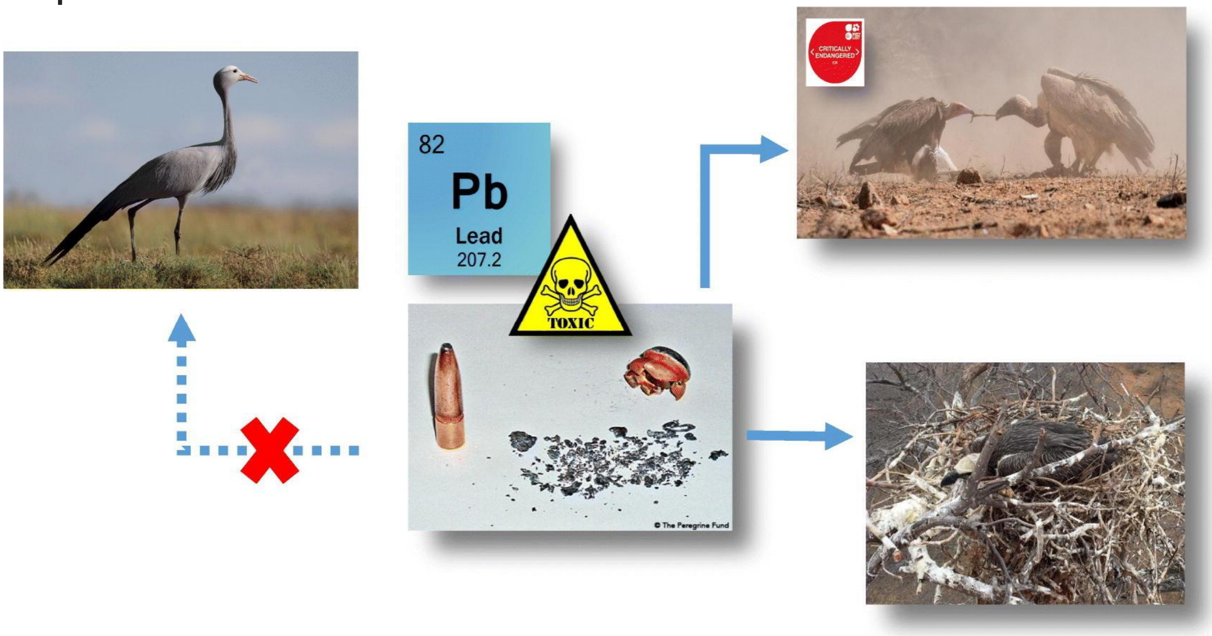
• Hunters are urged to switch to non-lead alternatives.

Abstract

Poisoning, including secondary lead poisoning, is cited as the single most important cause of vulture mortalities in Africa. To evaluate the prevalence of lead poisoning among South Africa's *Gyps* vultures compared to other, non-scavenging birds, we obtained blood and bone samples from Cape (*Gyps coprotheres*) and White-backed (*G. africanus*) vultures. We found that 66% of White-backed Vultures (n = 110, including 85 nest-bound chicks sampled at Dronfield Nature Reserve) and 80% of Cape Vultures (n = 15) had blood [Pb] in excess of 10 µg/dL, the upper limit of background exposure. Average blood [Pb} were 15.4 µg/dl and 29.7 µg/dl for White-backed and Cape vultures, respectively. Bone samples revealed that 12% of White-backed Vultures (n = 18) and 9% of Cape Vultures (n = 75) suffered from subclinical to severe clinical lead poisoning upon their deaths. By contrast, none of the 40 blood, bone or liver samples obtained from non-scavenging bird species were found to exceed background exposure levels. Our results suggest that, unlike non-scavenging birds, the scavenging lifestyle of *Gyps* vultures subjects them to lead poisoning on a regular basis. Had environmental sources of lead (e.g., dust) been the source of the lead poisoning at the White-backed Vulture breeding colony at Dronfield, all the chicks would have displayed similar blood lead concentrations. Instead the values ranged from barely detectable to very high, leading us to conclude that metallic lead fragments regurgitated by parents during feeding are responsible for the elevated lead levels in some of the chicks at this site. We conclude the likely source of these particles to be fragments of lead ammunition embedded in the carcasses of hunted animals. These results add to a growing body of evidence underscoring the threat posed by the use of lead ammunition and its potential role in the declines of vultures and other scavenging taxa.

Key words: vulture, lead, blood, bone, chick, ammunition

Graphical abstract



1 Introduction

Vultures are regarded as one of the most threatened avian functional guilds globally, and their populations have undergone precipitous declines in both Asia and Africa in recent decades (Botha et al., 2017; Buechley and Şekercioğlu, 2016; McClure et al., 2018; Ogada et al., 2012). In Asia, vulture declines have been linked, amongst others, to the consumption of cattle carcasses treated with the veterinary drug diclofenac (Oaks et al., 2004; Pain et al., 2003). Although habitat loss, decreased food availability, human disturbance, harvesting for belief-based use and collisions with and electrocutions by energy infrastructure are major contributing factors, poisoning, including secondary lead poisoning, has been cited as the single most important cause of vulture mortalities in Africa (Botha et al., 2017; Buechley and Şekercioğlu, 2016; Ogada et al., 2012; Virani et al., 2011).

Numerous studies have highlighted the prevalence of lead poisoning among large raptors that are either complete or partial scavengers, including Golden Eagle (*Aquila chrysaetos*; Wayland, Neugebauer and Bollinger, 1999; Madry *et al.*, 2015), Bald Eagle (*Haliaeetus leucocephalus*; Neumann, 2009; Bedrosian, Craighead and Crandall, 2012), California Condor (*Gymnogyps* *californianus*; Gwiazda *et al.*, 2006; Finkelstein *et al.*, 2012), Andean Condor (*Vultur gryphus*; Lambertucci *et al.*, 2011; Wiemeyer *et al.*, 2017), Steller's Sea Eagle (*H. pelagicus*; Ishii *et al.*, 2017), White-tailed Sea Eagle (*H. albicilla*; Helander *et al.*, 2009; Ishii *et al.*, 2017), as well as Black (*Coragyps atratus*) and Turkey vultures (*Cathartes aura*; Behmke, Mazik and Katzner, 2017). Multiple studies in Europe and the Middle East have also revealed widespread lead poisoning in Old World vultures, particularly Bearded (*Gypaetus barbatus*), Griffon (*Gyps fulvus*) and Egyptian Vulture (*Neophron percnopterus*; Garcia-Fernandez *et al.*, 2005; Horowitz *et al.*, 2014; Berny *et al.*, 2015; Carneiro *et al.*, 2015; Ganz *et al.*, 2018).

The pathophysiology of lead poisoning primarily involves inhibition of the cellular function of Ca^{2+} and consequent interference with neurotransmitter systems and synaptogenesis, as well as disruption of the blood brain barrier and decreasing total brain volume with chronic exposure (Holstege et al., 2015; Mason et al., 2014; Stewart et al., 2006). Among birds, lead could potentially impact every organ system in the body, most notably the central nervous and haematological systems (Eisler, 1988; Pokras and Kneeland, 2008). Acute exposure at extreme levels can lead to mortality, while chronic exposure can cause a variety of latent symptoms, including increased lethargy, decreased foraging ability, anorexia, decreased spatial awareness, breast-muscle atrophy, loss of strength and coordination, drooping wings, decreased reproductive success and depressed immune response (Franson and Pain, 2011; Garcia-Fernandez et al., 2005; Haig et al., 2014).

Long-lived, slow-breeding taxa such as large raptors have naturally low population numbers and the loss of just a few individuals can negatively affect population viability (Carpenter et al., 2003). Since Asian and African vultures continue to experience population declines due to factors such as poisoning, habitat loss and collision and electrocutions by energy infrastructure (Botha et al., 2017; Pain et al., 2009) the insidious effects of unintentional lead poisoning have the potential to be devastating. Not only could they reduce the breeding success of these already slow-breeding species, but could also make individuals more vulnerable to diseases and prone to collisions with energy infrastructure on account of compromised neurological functioning (Kelly and Kelly, 2005).

Vultures, like all vertebrates, obtain lead mainly through ingestion and inhalation (ATSDR, 2007). Lead particles may be inhaled as part of airborne dust, or from feathers during preening. One of the main causes of unintentional poisoning in birds is believed to be the inadvertent ingestion of metallic lead particles from the carcasses and/or gut piles of animals shot with lead ammunition (Buechley and Şekercioğlu, 2016). Ingested particles of lead are wholly or partially dissolved in the highly acidic stomach lumen and the resultant toxic lead salts are absorbed into the bloodstream (Franson and Pain, 2011). In Old and New World vultures this process is facilitated by their unusually acidic stomachs which, in some species, can reach a pH as low as 1.0 (Houston et al., 1975; Roggenbuck et al., 2014). The bloodstream transports lead to organs (most notably the liver and kidneys) and bone (Jenni et al., 2015), where it accumulates over the bird's lifetime (Fisher et al 2006). Although lead in bone is relatively immobile, its absorption and release accelerates in the medullary bone of egg-laying females (Franson and Pain, 2011), a phenomenon that can complicate the dynamics between lead in blood and lead in bone (T. Katzner, pers. comm.).

It should also be noted that, because the half-life of lead in blood is approximately two weeks (Franson and Pain, 2011), blood [Pb] integrates lead exposure over only a short period. In other words, low blood [Pb] at the time of sampling, does not necessarily mean that they have not experienced more severe poisoning in the past (Burger and Gochfeld 2001; Clarkson et al. 1988). A single exposure event may result in severe poisoning (or even mortality), but chronically assimilating sub-lethal amounts of lead may result in lowered reproductive success and/or higher mortality (Pain et al., 2009), which may affect a higher proportion of the population than those individuals that die from acute poisoning (Jenni et al., 2015).

Another possible avenue of lead exposure is biomagnification, whereby pollutants become more concentrated with increasing trophic level (Franson and Pain, 2011). Although an animal can bioaccumulate lead in some of its tissues over its lifetime, biomagnification of lead is minimal (ATSDR, 2007; Eisler, 1988; Farag et al., 1997; Szefer, 1991; USEPA, 2006), on account of its low trophic bioavailability (i.e. the fact that most lead is sequestered in calcified tissues such as bone, and is rarely found in the muscle tissue and organs favoured by predators) and a sequential Ca^{2+} biopurification process lowering [Pb]/[Ca^{2+}] ratios stepwise up the food chain (Mager, 2012; Patterson, 1980). It has been found that, in general, [Pb] is lower in predators than their prey, since rapid excretion occurs and absorption of metals across the gut tends to be incomplete (Fowler, 1982; Luoma, 1983; Szefer, 1991). Studies on the distribution of heavy metals in the body tissues of an antelope, Springbok (*Antidorcas marsupialis*), showed 97% of liver samples, 59% of kidney samples and all muscle samples to be below the detection limit (Magwedere et al., 2013). White-backed Vultures mostly feed on muscle and viscera, generally ignoring the skin and bones (Piper, 2005a) and even though they may consume the former tissues in large quantities, the negligible contribution of biomagnification could not account for their elevated and, in some cases, extreme blood [Pb].

The impact of lead poisoning on the vultures of southern Africa has received increased attention in recent years, with elevated lead levels found in Cape (*Gyps coprotheres*) and White-backed (*G. africanus*) vultures (Kenny et al., 2015; Naidoo et al., 2017), as well as the region's Critically Endangered subspecies of Bearded Vulture (Krüger and Amar, 2018). With the exception of Southern Ground-Hornbills (*Bucorvus leadbeateri;* Koeppel and Kemp, 2015), no studies to date have quantified lead levels in the region's non-scavenging bird species. Here, we combined an evaluation of the blood lead concentrations found in free-flying birds and developing chicks with an investigation into the levels of lead sequestrated in the bones of South Africa's *Gyps* vultures. We compared lead concentrations in vulture tissues with those in samples obtained from a wide range of raptor and other terrestrial bird species in order to evaluate the significance of lead concentrations found in vulture species, and propose a possible source of the lead poisoning.

2 Materials and Methods

2.1 Sampling locations

Blood and bone samples were obtained from Cape and White-backed vultures at 13 sites across South Africa, in all provinces except Gauteng, Western Cape and Northwest (Figure 1).

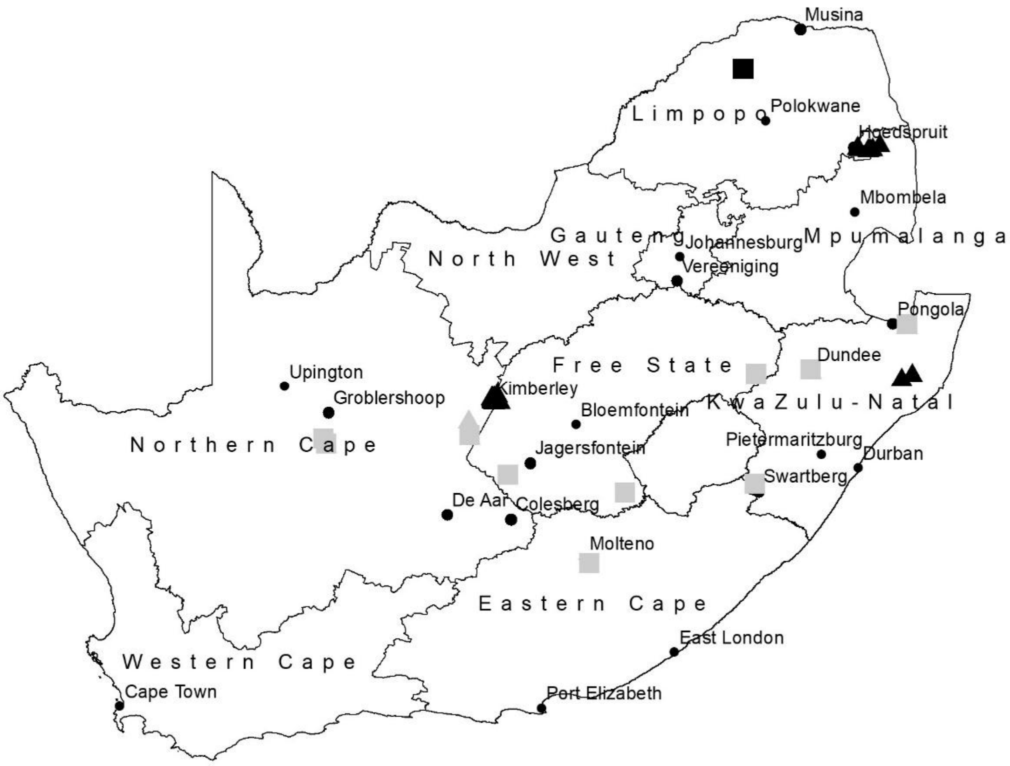


Figure 1: Locations in South Africa where blood (black) and bone (grey) samples were collected from White-backed (▲) and Cape (■) vultures.

2.1.1 Blood samples

Free-ranging White-backed and Lappet-faced vultures, including juveniles and adults, were trapped and sampled at Hluhluwe-Imfolozi Park (28.312° S; 31.862° E) in northern KwaZulu-Natal Province, South Africa, between September 2017 and February 2018. White-backed Vultures were also sampled at Hoedspruit Airforce Base (24.370° S; 31.045° E) and Timbavati Game Reserve (24.407° S; 31.314° E) near Hoedspruit in Mpumalanga Province, South Africa (September 2017). In all cases the birds were trapped using foot-nooses arranged around baited carcasses (as described in Monadjem *et al.*, 2018) and restrained by experienced handlers.

Unfledged White-backed Vulture chicks were sampled at Dronfield Nature Reserve (28.618° S; 24.809° E) near Kimberley (Northern Cape Province) in October 2016 and October 2017, and at Hoedspruit Air Force Base (Mpumalanga Province) during September 2017. Nests at Dronfield were accessed using an extension ladder, carefully placed in order to protect the integrity of the nest. Chicks were eased into a rubber hold-all attached to a rope and lowered to the ground. At Hoedspruit Air Force Base the height of the nest trees precluded the use of a ladder, so a cherry-picker was used to lift the handler up to the nest. Once a chick was removed from its nest it was brought down for processing and returned to its nest by the same route. In all the above cases blood samples were collected incidentally to scientifically coordinated ringing and tagging events. In total 110 White-backed Vultures were sampled, consisting of 19 free-flying adults and 91 unfledged chicks. Existing lead exposure may have made the free-flying birds more vulnerable to capture, which in turn could have affected the randomness of the results. Since existing lead exposure would not have made certain nest-bound chicks more vulnerable to capture, we consider the samples taken from chicks to be random.

Blood samples from Cape Vultures (mostly juveniles) were obtained from the breeding colony at Blouberg Nature Reserve (22.944° S; 29.147° E) in northern Limpopo between May 2017 and January 2018. During heavy rains recently fledged juveniles struggle to fly, often landing in the cattle kraals (i.e. enclosures) of local communities where they are easy to catch. Blouberg Nature Reserve runs a project for their retrieval, so that the birds can be dried and allowed to regain their strength. Samples were taken a week after a bird's arrival at the rehabilitation centre, when it was tagged and ringed prior to release. We acknowledged that blood [Pb] may have declined in the time between capture and sampling. Additionally, as lead poisoning could have made these birds more vulnerable to capture, we do not regard this as a random sample.

Blood samples were obtained from non-vulture species brought in for rehabilitation at the Johannesburg Wildlife Veterinary Hospital (Gauteng Province). These included Blue Crane

(*Anthropoides paradiseus),* Jackal Buzzard (*Buteo rufofuscus*), Spotted Eagle-Owl (*Bubo africanus*), Secretarybird (*Sagittarius serpentarius*), European Honey-Buzzard (*Pernis apivorus*) and African Fish-Eagle (*Haliaeetus vocifer)*. A blood sample was also obtained from a Verreaux's Eagle-Owl (*Bubo lacteus*) chick resident at the Dronfield vulture colony near Kimberley during the 2016 sampling season, and from free-flying Pale Chanting Goshawk (*Melierax canorus*) and Jackal Buzzard trapped for ringing purposes near De Aar (Northern Cape Province) in May 2017.

During processing the birds were kept in a recumbent position with their heads covered by a cloth. Using a disposable needle and syringe, a blood sample of at least 0.5 mL was drawn from either the tarsal vein on the leg or the brachial vein on the underside of the wing and transferred to a purple-topped, certified lead-free, 250-500µl EDTA tube (Kenny et al., 2015; Wiemeyer et al., 2017). The sample was carefully swirled to ensure thorough mixing with the EDTA medium and refrigerated or frozen until such time as it could be couriered (on ice) to the laboratory for testing. Based on the minimum blood sample requirement of 0.5 mL (imposed by laboratory requirements), birds weighing less than 832g were excluded from the study. The method described above was approved by the BirdLife South Africa Ethics Committee (refs. 2016/04/B and 2016/06/B), the Animal Ethics Committee of the University of Pretoria (ref. EC012-17) and the SANBI Research Ethics and Scientific Committee (ref. P18/41).

2.1.2 Bone samples

Whole/partial carcasses (partially or completely decomposed) from birds killed through collisions with energy infrastructure such as power lines and wind turbines were collected at Molteno (Eastern Cape) and De Aar (Northern Cape). These included Cape, Lappet-faced and White-backed Vulture, Blue Crane, Ludwig's Bustard (*Neotis ludwigii*), Kori Bustard (*Ardeotis kori*), Verreaux's Eagle (*Aquila verreauxii*) and Booted Eagle (*Hieraaetus pennatus*). A White-backed Vulture was also retrieved near Mokala National Park (Northern Cape), where it had drowned in a farm dam.

Ezemvelo KZN Wildlife contributed a number of whole/partial leg samples to the study. These were collected from Cape and White-backed vultures that had died at mass poisoning events near Swartberg (southern KwaZulu-Natal Province), Pongola (northern KwaZulu-Natal Province) and on farms near the Free State/KwaZulu-Natal provincial border. Legs were severed at the hip socket and refrigerated and/or frozen.

Unless otherwise stated, the following methods follow those of Hetter *et al.* (2008). Studies on Common Eider (*Somateria mollissima*) and American Woodcock (*Scolopax minor*) have shown that bone lead is not deposited uniformly throughout the avian body (Ethier et al., 2007). In order to ensure consistency, the proximal epiphyses of a femur was targeted as the main sampling area during this study. In cases where the femur was not available, the proximal end of the tibiotarsus was used, and failing that the head of a humerus.

Feathers, skin and excess muscle were removed from fresh bone samples before they were covered in compost and left until all soft tissues had decomposed. Stainless steel, disposable scalpels (replaced between each sample) were subsequently used to scrape off any remaining muscle and/or tendons, before the bone was wiped clean using distilled water and 70% ethanol. The proximal epiphyses was removed from the bone shaft using a hammer and stainless steel chisel, before it was crushed into smaller fragments. In order to ensure homogeneity, each sample was weighed, then dried at 60°C for 48 hours, or until a constant mass was reached (van Wyk et al., 2001). The sample was subsequently ground into a fine powder using a coffee grinder fitted with a stainless steel bowl and blades, and decanted into a sterile urine specimen jar. As [Pb] may differ between the cortical and trabecular bone (Hetter et al., 2008), the bone lead level measured would, at best, be the average concentration of the homogenised sample. After each sample excess bone powder was removed from the grinder using a Ryobi dust blower, before the bowl and blades were wiped clean with ultra-pure water and 70% ethanol. It was therefore inevitable that some sample loss occurred. Sample grinding, as well as cleaning of the grinder, occurred under a fume hood. A dust mask was worn throughout the grinding and cleaning process and powder-free latex gloves, worn at all times, were replaced between each sample.

2.1.3 Liver samples

Liver samples were obtained from Raptor Rescue, a rehabilitation unit at The African Bird of Prey Sanctuary near Pietermaritzburg (KwaZulu-Natal). Samples were obtained from birds that were euthanised between 2014 and 2017. Once a carcass had thawed sufficiently the liver was removed using a stainless steel scalpel (replaced between each sample). Each organ was placed into a sterilised urine specimen jar and frozen at approximately -20 °C. Species sampled included Peregrine Falcon (*Falco peregrinus*), Lanner Falcon (*F. biarmicus*), African Goshawk (*Accipiter tachiro*), Jackal Buzzard, African Harrier-Hawk (*Polyboroides typus*), Spotted Eagle-Owl, Western Barn Owl (*Tyto alba*) and Black Sparrowhawk (*A. melanoleucus*). Samples from birds raised in captivity were not included in the analyses. An Ovambo Sparrowhawk (*A. ovampensis*) was sourced from Fairlands (Gauteng), where it had died of unknown causes.

2.2 Lead analyses

The blood samples collected at Dronfield in 2016 were analysed using Graphite Furnace Atomic Absorption Spectroscopy (GFAAS) at the National Institute for Occupational Health, a laboratory accredited by the South African National Accreditation System (SANAS), in Johannesburg, South Africa (accreditation no. M0276). All other blood samples, as well as the bone and liver samples, were analysed using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) at V&M Analytical Toxicology Services (Pty) Ltd, a SANAS-accredited laboratory in George, South Africa (accreditation no. T0610).

Prior to analysis samples were digested overnight at 40°C in concentrated nitric acid (HNO_3), after which they were brought up to volume using ultra-purified water. Internal standards, i.e. indium, gallium and yttrium, were added at various stages of the analyses in order to facilitate accurate quantification. Non-matching calibration was used to standardise the machines and certified lyophilised urine or whole blood (human), obtained from Recipe Clincheck, were used as controls.

The Octapole Reaction System (ORS3) of the 7700x ICP-MS was operated in helium collision mode (He mode) for all analytes and all samples. An Agilent 7700x ICP-MS with a Micromist nebulizer was used throughout. Standard operating conditions were applied during sample analysis (plasma mode: normal, robust; RF forward power: 1550 W; sampling depth: 8 mm; carrier gas flow: 1.07 L/min; dilution gas flow: 0 L/min; spray chamber temperature: 2°C; extraction lens 1: 0 V; kinetic energy discrimination: 4.5 V; He cell gas flow: 4.3 mL/min).

2.3 Data interpretation

Franson and Pain (2011) define background lead exposure in blood for members of the order Falconiformes, which includes the Old World vultures, as <20 µg/dL. Studies have indicated inhibition of δ-ALAD, an enzyme that plays an integral role in haemoglobin production, in European raptors for blood lead levels as low as 5 µg/dL (Gómez-Ramírez et al., 2010; Martínez-López et al., 2004) and for waterbirds as low as 6 µg/dL (Martinez-Haro et al., 2011). Similar studies on Griffon Vultures *Gyps fulvus* have indicated a 15% δ-ALAD inhibition and increased oxidative stress for blood lead levels as low as 10 µg/dL (Espín et al., 2014). Although it has been suggested that *Gyps* vultures may be more tolerant to lead (Espín et al., 2014; Garcia-Fernandez et al., 2005), the sub-lethal effects on the genus are poorly understood and should not be under-estimated. In view of this, a conservative approach was adopted, with background exposure in blood considered as <10 µg/dL [in line with Finkelstein et al*.* (2012) and Garbett et al*.*, (2018)]. The interpretation levels of blood, bone and liver [Pb] are set out in Table 1. If required and for comparison purposes, all [Pb] values referenced from previous studies were converted to the following units using the conversion factors proposed by Franson and Pain (2011): µg/dL (blood), µg/g dry weight (bone) and µg/g wet weight (liver).

Table 1: Interpretation of [Pb] in different tissue types of members of the order Falconiformes, adapted from Wiemeyer et al. *(2017) and Franson and Pain (2011).*

Range	Interpretation
Blood (µg/dL)	
<10	Background
10 – 20	Mild to moderate subclinical effects
20 – 50	Significant subclinical effects
50 – 100	Clinical poisoning
>100	Severe clinical poisoning
Bone (µg/g dw[a])	
<10	Background
10 – 20	Subclinical to clinical poisoning
>20	Severe clinical poisoning
Liver (µg/g ww[b])	
<2	Background
2 – 6	Subclinical poisoning
6 – 10	Clinical poisoning
>10	Severe clinical poisoning

[a]dw = dry weight; [b]ww = wet weight

2.4 Statistical analyses

All statistical analyses were carried out in the R 3.5.1 (R Core Team, 2018) environment, using R Studio 1.1.463 (RStudio, Inc.). All blood and bone Pb concentrations were log_{10}-transformed to meet assumptions of normality (Shapiro-Wilk test). Assumptions of homoscedasticity were confirmed using Levene's tests implemented in the R package *lawstat* (Gastwirth et al., 2017). To compare blood and bone [Pb] between vultures and non-scavenging species, we fitted linear models with blood [Pb] or bone [Pb] as the response variable and taxonomy ("vultures" or order for non-scavenging species) as a predictor in the R package *nlme* (Pinheiro et al., 2007). To compensate for skewness, the geometric mean was employed in all instances. As all values fell above detection limits, statistical methods related to left-censored data were not employed (Helsel, 2012). A lack of geographical overlap between blood samples taken from chicks and adults, and the absence of bone samples from chicks, meant that age class comparisons were not feasible.

3 Results

3.1 Vulture species

Gyps vultures had elevated blood and bone [Pb] throughout their South African range (Table 2 and 3). Lead concentrations varied from background to severe clinical poisoning in both White-backed and Cape vultures (Figure 2 and 3). Four of the 15 Cape Vultures sampled at Blouberg Nature Reserve, with blood [Pb] ranging from 37.9 to 109.2 µg/dL, are known to have died from a variety of causes, including drowning and collisions. We do not consider the Cape Vulture samples taken at Blouberg Nature Reserve to be representative of this population, as their elevated blood [Pb] could have made them more vulnerable to capture. Blood [Pb] was generally higher and more variable among vultures compared to those of other, non-scavenging taxa (Table 5).

3.2 Comparison with non-vulture species

All non-vulture species displayed blood, bone and liver [Pb] below background levels (Table 4). The mean blood [Pb] of White-backed Vultures was 15.4 ± 21.0 µg/dL, and that of Cape Vultures was 29.7 ± 37.6 µg/dL (Figure 4). Mean bone [Pb] of White-backed Vultures was 6.8 ± 6.8 µg/g and that of Cape Vultures was 2.8 ± 4.0 µg/g (Figure 4). Both blood [Pb] and bone [Pb] varied significantly with taxonomy (blood: $F_{3,143} = 26.967$, $P < 0.001$; bone: $F_{3,132} = 4.204$, $P = 0.007$). Although the small sample sizes for some of the orders precluded reliable post hoc tests, the conclusion that vultures had significantly higher blood [Pb] and bone [Pb] is supported by the clear differences evident in Figure 4.

Table 2: Blood [Pb] (µg/dL) in White-backed, Cape and Lappet-faced vultures in South Africa.

Species	Year	Province	Age class	n	Mean	SD	Min	Max
Vulture, White-backed								
Dronfield Nature Reserve	2016/17	Northern Cape	Chicks	85	14.4	17.7	2.4	84.9
Hoedspruit/Timbavati	2017	Mpumalanga	Chicks – Adults	10	14.7	12.8	5.4	47.0
Hluhluwe-Imfolozi Park	2017	KwaZulu-Natal	Juveniles-Adults	15	23.1	35.3	6.0	134.9
Total				110	15.4	21.0	2.4	134.9
Vulture, Cape								
Blouberg Nature Reserve	2017/18	Limpopo	Juveniles-Adults	15	29.7	37.6	1.1	109.2
Vulture, Lappet-faced								
Hluhluwe-Imfolozi Park	2018	KwaZulu-Natal	Juveniles-Adults	2	7.6	-	3.2	12.0

Table 3: Bone [Pb] (µg/g dw) in White-backed, Cape and Lappet-faced vulture carcasses collected in various regions of South Africa. The age class of the samples could not be determined.

Species	Sample size	Mean	SD	Min	Max
Vulture, White-backed	18	6.8	6.8	0.6	27.5[a]
Vulture, Cape	75	2.8	4.0	0.2	17.6
Vulture, Lappet-faced	4	1.1	0.3	0.6	1.5
Total	97				

[a]This individual was recovered from a water reservoir on a farm near Mokala National Park, where it had drowned.

Table 4: Lead concentrations found in blood, bone and liver samples from a variety of non-vulture species, including other members of the Accipitriformes, and members of the Falconiformes, Gruiformes, Otidiformes and Strigiformes. For each tissue type, all values were found to be within background levels. Information on diet from species accounts in Hockey, Dean and Ryan (2005).

Species		Blood		Bone		Liver	Major diet items
	n	Mean (µg/dL)	n	Mean (µg/g dw)	n	Mean (µg/g ww)	
Background exposure*		<10		<10		<2	
Bustard, Kori			1	1.2			Invertebrates, small vertebrates, carrion (roadkill and fire casualties) and vegetable matter.
Bustard, Ludwig's			1	0.7			Invertebrates, small vertebrates and vegetable matter.
Buzzard, European Honey	4	5.6					Insectivorous.
Buzzard, Jackal	4	2.4			1	0.03	Scavenges mainly at roadkill, also sheep placentae. Hunts mainly small mammals, and some birds and reptiles.
Crane, Blue	1	3.5	3	2.6			Mostly vegetarian; also insects, worms, crabs, fish, frogs, reptiles and small mammals.
Eagle, African Fish	1	0.2					Fish, birds, reptiles and small mammals.
Eagle, Booted			1	1.5			Mainly birds; also lizards and rodents.
Eagle, Verreaux's'			1	0.5			Rock Hyrax.
Falcon, Lanner					1	0.2	Birds; also small mammals, reptiles and insects.
Falcon, Peregrine					3	0.04	Birds; also birds and occasionally insects.
Goshawk, African					2	0.04	Birds; also small mammals and reptiles.
Goshawk, Pale Chanting	2	1.0					Small mammals, birds, reptiles and insects.
Hawk, African Harrier-					1	0.05	Birds, small mammals, reptiles; may eat carrion.
Owl, Spotted Eagle-	6	1.3			2	0.02	Rodents, shrews, small birds.
Owl, Verreaux's Eagle-	1	4.7					Birds, insects, small mammals and young of larger mammals.
Owl, Western Barn					1	0.01	Small mammals, birds; also frogs, lizards and insects.
Secretarybird	1	2.0					Wide variety of animal prey, but no carrion.
Sparrowhawk, Black					1	0.2	Mainly birds.
Sparrowhawk, Ovambo					1	0.04	Birds.
Mean	20	2.0	7	0.8	13	0.03	

*Background exposure level for each specific tissue type. Based on Franson and Pain (2011).

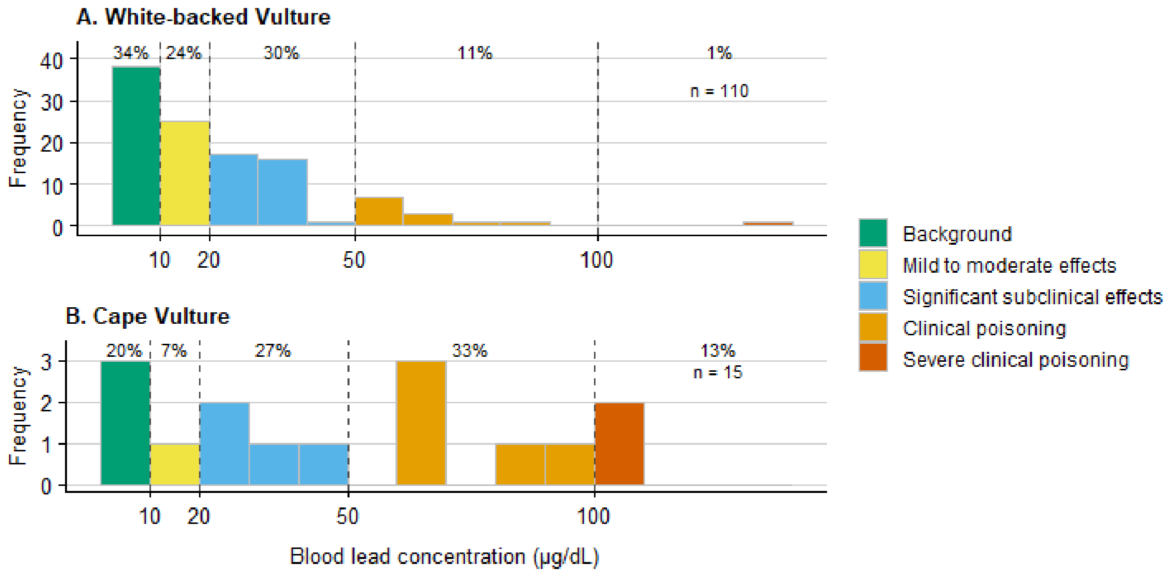


Figure 2: The distribution of blood lead concentrations (µg/dL) indicates that 66% of White-backed (A) and 80% of Cape (B) vultures had blood [Pb] above 10 µg/dL, the upper limit for background exposure.

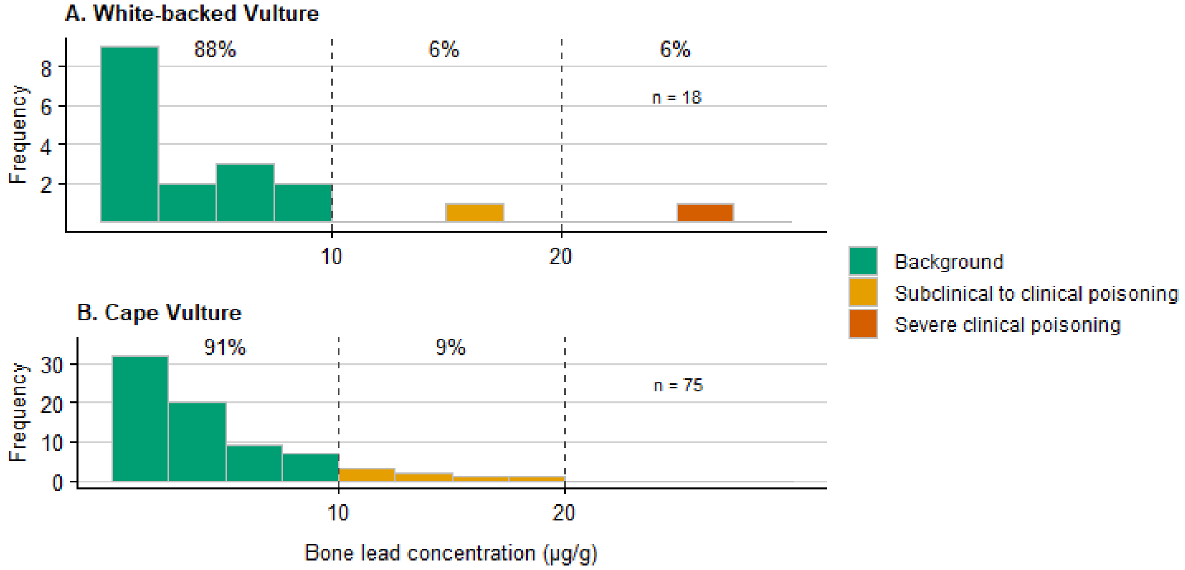


Figure 3: The distribution of bone lead concentrations (ug/g) indicates that, upon their deaths, 12% of White-backed (A) and 9% of Cape (B) vultures had body lead burdens consistent with subclinical to severe clinical lead poisoning.

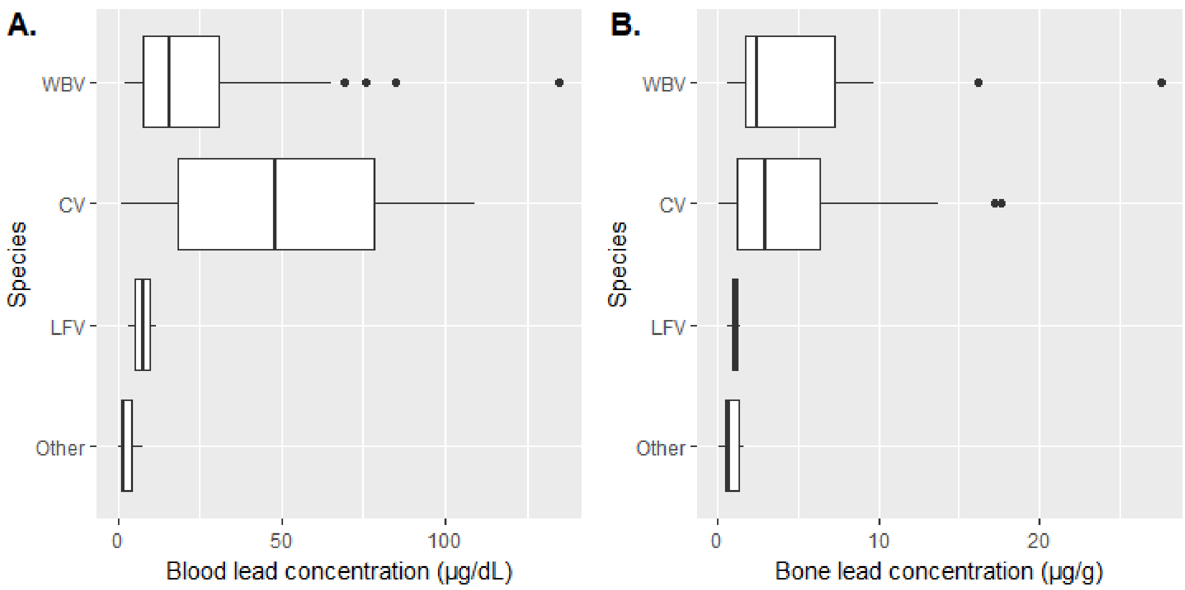


Figure 4: Blood (A) and bone (B) lead concentrations of White-backed (WBV), Cape (CV) and Lappet-faced (LFV) vultures, as well as those of other, non-vulture species. Outliers indicate birds with extreme [Pb], exceeding Q_3 by more than 1.5 times the interquartile range

4 Discussion

4.1 Blood lead concentrations

Our data confirm that South Africa's White-backed Vulture population faces exposure to above-background levels of lead poisoning throughout its regional range. It is concerning that 66% of the birds sampled had blood [Pb] > 10 µg/dL (upper limit to background exposure), especially considering that most of the samples were taken from unfledged chicks. The prevalence of lead exposure we documented are broadly consistent with those reported by Naidoo *et al.* (2017), but more than double that found by Garbett *et al.* (2018) in Botswana (Table 5), suggesting that lead exposure in South Africa's White-backed Vultures far exceeds that of its neighbouring country.

Our data for White-backed Vulture chicks at Dronfield provide novel insights into the potential sources of lead. All the chicks are presumably equally exposed to environmental lead from airborne dust or ingestion when preening their feathers. If widespread environmental exposure was the main cause of lead toxicosis in these chicks, all of which are confined to a 120 km^2 reserve, we would expect similar blood [Pb] among individuals (consistent with Katzner et al., 2018). Instead, blood [Pb] displayed a distinctly right-skewed distribution (Figure 2A), which is typical of lead poisoning involving exposure to pure, metallic lead particles (Franson and Pain, 2011; Scheuhammer and

Norris, 1996). Since it is unlikely that these nest-bound chicks are encountering fragments of lead in their immediate environment (i.e. the nest), then the only other possible avenue of exposure is via their parents. Slivers of meat collected at carcasses could have been ingested along with dust and soil at the point of origin, but, as only lead in metallic form is able to induce such high blood lead levels (Garcia-Fernandez et al., 2005), it is far more plausible that fragments of pure metallic lead, embedded within the carrion on which they were feeding, were responsible for the elevated lead levels found in their chicks. Interestingly, a single Verreaux's Eagle-Owl chick resident within the Dronfield breeding colony had blood [Pb] of 4.67 µg/dL, within background exposure values for the vultures. The above patterns were echoed by blood [Pb] in chicks at Hoedspruit Airforce Base (Mpumalanga), albeit with a much smaller sample size.

Table 5: Comparative statistics between this study and previous studies conducted on vulture species both in southern Africa and abroad.

Species	Country	>10 µg/dL	Mean blood [Pb] (µg/dL)	Mean bone [Pb] (µg/g)
Vulture, White-backed				
This study	South Africa	66%	15.4 ± 21.0	6.8 ± 6.8
Naidoo et al. (2017)	South Africa	55%	16 – 28	
Garbett et al. (2018)	Botswana	32%	10.7 ± 11.0	
Vulture, Cape				
This study	South Africa	80%	29.7 ± 37.6	2.76 ± 4.0
Naidoo et al. (2017)	South Africa	40%	11 – 14	
Vulture, Bearded				
Krüger and Amar (2018)	South Africa / Lesotho		0.6 ± 0.8	11.8 ± 8.3
Vulture, Griffon				
Garcia-Fernandez et al. (2005)	Spain		43.07	
Carneiro et al. (2015)	Portugal		29.7 ± 13.2	
Condor, Andean				
Wiemeyer et al. (2017)	Argentina	36%	15.5 ± 21.2	23.1 ± 31.4

Of great concern are the sub-lethal effects lead poisoning might be having on the neurological and skeletal development of unfledged chicks. Of the 85 birds sampled, 52% displayed lead levels consistent with mild/moderate to significant subclinical effects. These levels would, in all probability, not directly lead to mortality, but result in behavioural impairments that might reduce a bird's ability to avoid other causes of mortality (Scheuhammer and Norris, 1996). Leaving the nest with a significant lead burden may lower a juvenile vulture's chances of survival, making it less able to navigate effectively, fight for its share at carcasses or negotiate manmade infrastructure (Kelly and Kelly, 2005).

The mean blood [Pb] obtained from Cape Vultures at Blouberg Nature Reserve was significantly higher than the national mean reported by Naidoo *et al.* (2017), with 80% of samples having blood [Pb] in excess of 10 µg/dL (as opposed to 40%; Naidoo *et al.* 2017). It is concerning that four of the 15 Cape Vultures sampled at Blouberg are known to have subsequently died, with a fifth sustaining an injury that reduced it to permanent captivity. Since the fates of the remaining ten birds remain unknown, the mortality rate could be even higher, especially when considering that two of the birds whose fates are not known had blood lead levels of 86 ug/dL and 90 ug/dL respectively.

Although researchers generally consider blood [Pb] >100 ug/dL indicative of severe clinical poisoning, a number of studies have demonstrated significant inter- and intraspecific variation in tolerance among New World raptors (Bedrosian et al., 2012; Carpenter et al., 2003). Similar studies are lacking on Old World vultures, although the possibility exists that *Gyps* vultures display similar variability. This highlights the need to better understand the possible impact lead poisoning may have on a vulture's breeding success, its ability to negotiate manmade infrastructure, its foraging success and, ultimately, its lifespan. A better understanding is also required of the other factors, such as age, gender and general health, which may influence the susceptibility of *Gyps* vultures to lead poisoning.

4.2 Bone lead concentrations

The majority of White-backed and Cape Vulture bone samples had bone [Pb] within background exposure levels (<10 µg/g), with 9-10% displaying exposure levels consistent with subclinical, clinical or severe clinical lead poisoning (Figure 3). The mean bone [Pb] of White-backed and Cape Vultures were substantially lower than that found in southern Africa's Bearded Vulture population (Table 5).

Conversely, mean blood [Pb] found in six Bearded Vultures were substantially lower than those found in White-backed and Cape vultures in this study (Krüger and Amar 2018), leading these authors to conclude that, although southern Africa's Bearded Vultures do not show signs of acute or short-term exposure, they are nevertheless accumulating lead in bones over their lifetime. The opposite seems to hold for Cape and White-backed vultures, with individuals apparently dying from acute poisoning or side effects before lead can be sequestered in the skeleton (findings similar to Jenni et al., 2015).

Bearded Vultures have evolved a highly acidic gastric physiology for the digestion of bone and marrow; an adaptation that also favours the absorption of lead (Berny et al., 2015). Since South Africa's *Gyps* vultures favour organs and muscle tissue, the contribution of biomagnification to their body lead burden is likely minimal. Since most of the body's lead burden is stored in bone (Franson and Pain, 2011; Mager, 2012), there is a possibility that Bearded Vultures are more vulnerable to biomagnification of lead on account of their diet. This could explain the slow, sub-lethal accumulation of bone lead during their lifetimes. The apparent lack of acute exposure in Bearded Vultures implies that, unlike Cape and White-backed vultures, they are less likely to be exposed to fragments of metallic lead (such as spent ammunition), which usually becomes embedded in organs and muscle tissue rather than bone. It is also worth noting that the mean [Pb] of four Lappet-faced Vultures bone samples fell far below background exposure, potentially attributable to this species' preference for skin, ligaments and tendons and general avoidance of muscle and viscera (Piper, 2005b). Two blood samples from Lappet-faced Vultures also suggest lower exposure levels than *Gyps* vultures.

Even though [Pb] in the majority of bone samples fell within background exposure levels, it is concerning that bone [Pb] in ~10% of individuals exceeded background limits (Figure 3). This could be significant for species such as the White-backed Vulture that have declined precipitously in recent years as a result of multiple threats (Botha et al., 2017). Since calcium absorption and deposition are rapid in young birds, large amounts of lead will be deposited in the skeleton early in life, raising concerns about poor skeletal development and lead stores that may be remobilised during egg-shell formation and general calcium shortages (Franson and Pain, 2011).

4.3 Comparison with other species

Bone, liver and blood [Pb] in non-vulture species, including 16 raptors and three large terrestrial species, were all consistent with tissue-specific background exposure (Table 4). With just two exceptions, Kori Bustard and Jackal Buzzard, all sampled species rely on hunting as their major foraging mode. Although Kori Bustards and Jackal Buzzards occasionally supplement their diet with carrion, these are mostly obtained from fire casualties and/or roadkill (Allan, 2005; Allan and Osborne, 2005). Although increased [Pb] have been found in numerous species of non-scavenging raptors and members of the Gruiformes, these are mostly related to the ingestion of lead shot ingested by waterfowl, or the ingestion of lead shot as grit (Fisher et al., 2006; Martin et al., 2008; Pain et al., 2009).

Since mean blood and bone [Pb] of Cape and White-backed vultures were significantly higher than those of non-vulture species (Figure 4), indications are that vultures' obligate scavenging lifestyle is predisposing them to lead poisoning. Since Cape Vultures were sampled one week after capture, blood [Pb] would have decreased during their time in captivity. The inclusion of these data strengthen the conclusion, based on the significant differences between vultures and other taxa, that vultures' exposure levels are significantly higher. It may be beneficial for future studies to investigate facultative scavengers such as Yellow-billed Kite *Milvus parasitus* and Bateleur *Terathopius ecaudatus*, as well as White-headed *Trigonoceps occipitalis* and Hooded *Necrosyrtes monachus* vultures, whose lead burdens were not evaluated here. Animals that have died of natural causes such as predation are unlikely to be important sources of lead, as they are unlikely to contain the metallic lead particles that are required to induce the elevated [Pb] observed in this study. By contrast, the carcasses and gut piles of animals shot with lead ammunition are known to retain high densities of lead fragments, providing vultures with ample opportunity to encounter fragments of metallic lead (Craighead and Bedrosian, 2008; Hunt et al., 2004).

The overall patterns of lead exposure we have found among vultures in South Africa are broadly consistent with findings from other regions. Griffon Vultures (*Gyps fulvus*) in Portugal and Spain (Carneiro et al., 2015; Garcia-Fernandez et al., 2005) showed similar mean blood [Pb] to those of southern African species (Table 5), in both instances suggesting bullet lead fragments embedded in carcasses to be the probable source of lead. Griffon Vultures displayed blood [Pb] 5 – 16 higher than those of other, non-scavenging raptor species (Garcia-Fernandez et al., 1997; Martínez-López et al., 2004). Similar blood [Pb] was also found in the Andean Condor, a species phylogenetically distant to *Gyps* but occupying a similar ecological niche (Table 5; Wiemeyer *et al.*, 2017). Similarly to vultures in Botswana (Garbett *et al.* 2018), 36% of condors sampled had blood [Pb] > 10 µg/dL (Wiemeyer *et al.*, 2017. These studies reveal the global nature of the threat posed by lead to avian scavengers, compounding the diverse array of anthropogenic threats already faced by these birds.

5 Conclusion

We conclude that lead poisoning in southern Africa's *Gyps* vultures is a much larger problem than previously thought, affecting not only the adult population but also threatening the development of nest-bound chicks. As it is difficult to conceive of any other behaviour that could possibly expose vultures to metallic lead fragments, we argue that the major avenue of lead exposure involves fragments ingested while feeding on carcasses and/or gut piles of animals shot with lead ammunition. Removing the gut piles from the field could lower the risk of exposure, but may, in some areas, deprive vulture populations of an important source of food. Making the switch to lead-free ammunition would greatly reduce the risk of exposure, not only to wildlife, but also to humans who may inadvertently ingest lead particles embedded in game meat (Grainger Hunt et al., 2009). We also recommend the careful management of supplementary feeding sites, as inadequate scrutiny of carcasses may inadvertently expose scavengers to carcasses of animals killed with lead ammunition (L. van den Heever, pers. obs.).

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