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Abnormal lead exposure in globally threatened Cinereous vultures (*Aegypius monachus*) wintering in South Korea

Dong-Ha Nam · Doo-Pyo Lee

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Abstract Globally threatened Cinereous vultures (*Aegypius monachus*) regularly over-winter in South Korea, and they have frequently been found dead in their natural habitats. As one possible factor for their mortality, we investigated tissues for heavy metal contaminants along with necropsies on 20 dead Cinereous vultures. Severe emaciation was found in the survey, being associated with 19 of the deaths. Two of the 19 showed lesions suggestive of lead poisoning in the tissues; there was no indication of trauma, embedded shot, lead bullets in the stomach, or signs of electrocution in the specimens. Of 20 vultures, two showed lesions compatible with death from lead poisoning with 19.7 ppm dry weight (6.9 ppm wet weight) and 34.1 ppm dry weight (11.1 ppm wet weight), and 14 individuals had a potentially toxic level of lead with >6 ppm dry weight (about 2 ppm wet weight) in liver or kidney. The ingestion of lead-contaminated carcasses probably occurs along their migratory route. The possibility of lead exposure from the breeding site (Mongolia) or stopover area (China) should also be considered because some individuals are likely to die at or upon arrival. Our results suggest that most of the dead Cinereous vultures may be suffering from abnormally high lead exposure, indicating a potentially important cause of mortality in this endangered species.

Keywords Cinereous vulture · Mortality · Lead · Poisoning

D.-H. Nam
Center for Marine Environmental Studies (CMES), Ehime University, Bunkyo-cho 2-5, Matsuyama 790-8577, Japan
e-mail: dongha@umich.edu

Present Address:
D.-H. Nam
Department of Environmental Health Sciences, School of Public Health, University of Michigan, Ann Arbor, MI 48109-2029, USA

D.-P. Lee (✉)
Department of Biological Science, Honam University, Kwangsan-gu, Kwangju 506-714, South Korea
e-mail: dplee@honam.ac.kr

Introduction

Bioaccumulation of toxic substances has been associated with harmful effects on raptor populations (Wiemeyer et al. 1988; Newton et al. 1993) and heavy metals such as lead (Pb) are also still of concern for possible adverse effects to wild populations (Garcia-Fernandez et al. 2005). Raptors are frequently reported dying from Pb exposure through ingesting Pb-contaminated sources including directly through Pb projectiles or fragments from the ammunition used in hunting (Fisher et al. 2006). Although in recent years legislative regulation has imposed restrictions on the use of Pb shot for hunting (Fisher et al. 2006), Pb-related mortality of raptors has still been reported (Clark and Scheuhammer 2003). In South Korea, approximately 202 tons of Pb shot are used annually for hunting game birds and mammals and for clay target shooting each year (KME (Korean Ministry of Environment) 2003), without a voluntary or statutory ban on the use of Pb shot for hunting. Thus, the geographic distribution of Pb sources including spent Pb shot in South Korea is highly suspected to be widespread.

The Cinereous vulture (*Aegypius monachus*) is an endangered species (critically protected from 1973 in South Korea) and one of the rarest raptors in the world (BirdLife international 2004). Its global population is

estimated at between 7,200 and 10,000 pairs, with 1,700–1,900 pairs in Europe, and 5,500–8,000 pairs in Asia (BirdLife International 2004). While European countries have extensively carried out conservation projects on this species (Poirazidis et al. 2004; Morán-lópez et al. 2006; Hernández and Margalida 2008), much less information is available regarding the status, population trends, and bioaccumulation of toxic substances from Asia, where a predominance of the global population resides.

At least 1,000 individuals have been over-wintering in South Korea, with the largest population around the Korean Demilitarized Zone (DMZ) which provides a refuge to about 95% of the South Korean over-wintering population (KME and NMER (Korean Ministry of Environment and National Ministry of Environmental Research) 2004; Lee et al. 2006). While Cinereous vulture populations regularly over-winter in South Korea, they have frequently been found dead or moribund.

Some causes that appear to have contributed to the death of Cinereous vultures over-wintering in South Korea are starvation during migration and environmental contamination, but there have been no comprehensive investigations in their cause of death. Among raptor species, Pb poisoning has been well documented for Bald and Golden eagles with ~10% of their mortality (Wayland and Bollinger 1999; Fisher et al. 2006). This prompts us to determine causes of mortalities of all dead Cinereous vultures. Consequently, as one possible factor for their mortality, we conducted necropsies on dead Cinereous vultures and analyzed tissues for heavy metal contaminants that may have been involved in the cause of death.

Materials and methods

The study species mainly feed on carrion. It forms monogamous pair bonds. Its average clutch size is one and a single egg hatches after a 50–55 days incubation period. Between 2000 and 2003, 20 dead Cinereous vultures were submitted to the Korea Association for Bird Protection where necropsies were performed by veterinarians. All birds necropsied were immature (1–3 year-old) Cinereous vultures which are identified by plumage pattern. Necropsies involved an initial investigation of animal condition (emaciation based on adipose tissue with atrophy of pectoral and limb musculatures and carcass dehydration), physical appearance of the body (trauma, accident, electrocution, and shot), and anatomical approaches (embedded Pb shot pellets, Pb bullets or fragment in the digestive tract by dissection, and lesions suggestive of poisoning in the tissues). In the present study, severe emaciation was found in the most of necropsies, which are incorporated with lack of adipose tissue with atrophy of pectoral and limb musculatures. Following necropsies, frozen samples were sent to the Laboratory for Avian Biology in Honam University for chemical analysis.

Sub-samples of liver, kidney, and bone tissues were carefully prepared to avoid external contamination, and stored at −20°C until chemical analysis. All samples were dried separately in an oven at 80°C for 12 h until no further weight reduction occurred, and were homogenized in a glass Teflon homogenizer. About 1–3 g of tissue sample was then digested in the presence of a mixture of concentrated nitric, perchloric and/or sulfuric acids. Heavy metal concentrations (Pb, Cd, Zn, Cu, Fe, and Mn) were determined by the direct analysis of the digested samples (Nam et al. 2005) and/or a concentration step (Nam and Lee 2006) using flame atomic absorption spectrophotometry (Shimadzu AA-6400, Japan) wherever appropriate.

Quality control was assured by reference materials (DORM-2, National Research Council, Canada) in triplicate that were within the certified mean values (±15%) (Nam et al. 2005). Intervals of Pb residues are based on the reports by Franson (1996) and Clark and Scheuhammer (2003): >2.0–8.0 ppm wet weight (toxic Pb) and >8.0 ppm wet weight (compatible with death from Pb) in liver, >2.0–6.0 ppm wet weight (toxic Pb) and >6.0 ppm wet weight (compatible with death from Pb) in kidney. The approximate equivalent wet weight values were calculated based on average moisture content of liver (65.1%) and kidney (67.4%) of dead Cinereous vultures ($n = 20$). The conversion factors that we used were 2.87 for liver and 3.07 for kidney.

Results

Severe emaciation (lack of adipose tissue with atrophy of pectoral and limb musculatures) was found in the survey, being associated with 19 of the deaths. Two of the 19 showed lesions suggestive of poisoning; there was no indication of trauma, embedded shot, Pb bullets in the digestive tract, or signs of electrocution in the specimens examined. Residues of Pb, Cd, Zn, Fe, Mn, and Cu in liver, kidney, and bone tissues were analyzed and the results are shown in Table 1. Two out of 20 vultures had Pb levels with 19.7 ppm dry weight (6.9 ppm wet weight) and 34.1 ppm dry weight (11.1 ppm wet weight) suggestive of Pb poisoning, with evidence of a distended gall bladder and bile staining in their tissues; 14 individuals had a potentially toxic level of Pb (>6 ppm dry weight or >2 ppm wet weight); the remaining four vultures had Pb levels <6 ppm dry weight (<2 ppm wet weight) in their liver or kidney (Table 2). Concentrations of Cd, Zn, Fe, Mn, and Cu in tissues were generally within the range of healthy birds and thus do not appear to be diagnostic of toxicity by these

Table 1 Heavy metal concentrations (μg/g dry weight) in tissues of dead Cinereous vultures ($n = 20$)

	Pb	Cd	Zn	Fe	Mn	Cu
Liver						
Mean ± SD	10.5 ± 5.4	0.6 ± 1.0	122 ± 59	1,051 ± 422	6.3 ± 3.2	32.5 ± 17.3
Median	10.1	0.3	108	1005	6.2	30.0
Range	1.3–19.7	ND–4.6	49–337	380–1,715	0.6–11.8	7.8–74.2
Kidney						
Mean ± SD	10.6 ± 7.2	0.4 ± 0.4	76 ± 39	182 ± 120	4.1 ± 4.1	10.8 ± 5.2
Median	11.0	0.2	71	164	3.3	12.1
Range	2.2–34.1	ND–1.1	30–177	60–498	0.8–17.5	2.0–18.1
Bone						
Mean ± SD	8.3 ± 9.0	0.1 ± 0.1	118 ± 20	64 ± 34	4.6 ± 9.0	1.2 ± 1.0
Median	3.5	0.1	110	54	2.4	1.0
Range	1.0–29.0	ND–0.3	97–164	37 ± 157	0.6–38.1	0.2–4.0

The approximate equivalent wet weight values can be calculated based on average moisture content of liver (65.1%) and kidney (67.4%) of 20 dead Cinereous vultures

ND not detected

Table 2 Frequency of dead Cinereous vultures with levels of Pb associated with toxic effects and the ratios of liver or kidney Pb to bone Pb

	N	Toxic Pb	Compatible with death from Pb	Ratio of liver or kidney Pb to bone Pb (>1)
Liver[a]	20	16	0	14
Kidney[b]	20	14	1	15

Intervals of Pb residues are based on the reports by Franson (1996) and Clark and Scheuhammer (2003) to categorize severity of effects by Pb: >2.0–8.0 ppm wet weight (toxic Pb) and >8.0 ppm wet weight (compatible with death from Pb) in liver, >2.0–6.0 ppm wet weight (toxic Pb) and >6.0 ppm wet weight (compatible with death from Pb) in kidney

[a] Based on average moisture content of liver (65.1%) ($n = 20$)

[b] Kidney (67.4%) ($n = 20$)

heavy metals (see supporting information by Oaks et al. 2004).

Discussion

Mass mortality of Cinereous vultures in the winter of 2000–2001 was observed in Primorye, Russia, and the median concentrations (μg/g dry weight) of Pb (2.21 ppm), Cd (0.72 ppm), Zn (254 ppm), Fe (4,065 ppm), Mn (10.0 ppm), and Cu (77 ppm) in livers were found in 13 dead vultures (Kavun 2004). The level of heavy metals especially Pb in Cinereous vultures that migrate between Russia and northern China/western Mongolia was regarded as insignificant for their mortality (Kavun 2004). However, the Pb residues in liver (10.1 ppm dry weight or 3.5 ppm wet weight) and kidney (11.0 ppm dry weight or 3.6 ppm wet weight) of 20 individuals in this study are considered to be high.

The occurrence of Pb poisoning in dead Bald eagles (*Haliaeetus leucocephalus*) in the United States and Canada were 3–15%, with hepatic or renal Pb levels of >20 ppm (dry weight) (Reichel et al. 1984; Elliott et al. 1992). Of 57 White-tailed eagles (*H. albicilla*) found dead or moribund in Germany and Austria, 21–23% were suspected to have died of Pb poisoning (Kenntner et al. 2001). The lethal level of Pb may be related to species sensitivity with various biological and physiological factors, but the general guidance of Pb residues in Falconiformes has been established to categorize a number of effects by Pb (Franson 1996). Although this is based on results for another group of predators/scavengers (Falconiformes) not to Accipitriformes, it may be seen as the best comparison available. Moreover, both tissue Pb residues and necropsy observations of Pb-related lesions should be contemplated for a reliable diagnosis of Pb exposure on target species. In the present study, we found lesions suggestive of Pb poisoning in two dead Cinereous vultures with a distended gall bladder and bile staining of their tissues: one showed 19.7 ppm dry weight in liver and 6.8 ppm dry weight in kidney; the other showed 15.2 ppm dry weight in liver and 34.1 ppm dry weight in kidney. This suggests that two

individuals may have sufficient Pb residues in liver or kidney compatible with death from Pb, even if these threshold Pb levels are likely to be lower than those of other reported birds of prey.

The potential Pb exposure of dead Cinereous vultures is likely associated with their feeding habits as a result of ingestion of embedded Pb projectiles or fragments from the ammunition used in hunting in their preys. Extensive usage of Pb shot (about 202 tons annually) without legislative regulation in South Korea may also exacerbate the ecological risks of Pb exposure to this scavenger species, although we did not find direct consumption of Pb pellets in the ventriculus examined. A greater prevalence of high Pb exposure in Bald eagles occurred when the eagles were feeding largely on their prey during hunting season (Elliott et al. 1992; Wayland and Bollinger 1999). Since Cinereous vultures are generally over-wintering during the hunting season (November to February) in South Korea, many individuals are likely to experience high Pb exposure during that period (Fig. 1). The ingestion of embedded Pb projectiles or fragments from the ammunition used in hunting in their preys probably occurs along their migratory route (not far from the DMZ) because the higher Pb concentrations in tissues were observed in the late autumn, and because hunting activity is restricted within the DMZ. The ratios of hepatic Pb/bone Pb and kidney Pb/bone Pb are used as an indicator for exposure pattern of birds to Pb because the biological residence times of Pb are very different between soft tissues and bone due to differences in blood perfusion rates within these tissues (Jager et al. 1996; Church et al. 2006). In the present study, most individuals (70–75%) had hepatic or renal Pb concentrations greater than those in the bone (Table 2), suggesting a recent high exposure to Pb. Acute and chronic exposure to Pb can affect avian populations by altering reproductive success, behavior, immune response, and physiology (Burger and Gochfeld 2000; Fair and Ricklefs 2002), thus it is plausible that the recent elevated Pb exposure may be one of the significant sources of mortality to this globally threatened bird of prey.

Toward a comprehensive understanding of the migratory routes of Cinereous vultures, several young Cinereous vultures have been tracked from central Mongolia to South Korea (KCHA (Korean Cultural Heritage Administration) 2004). Based on the migratory routes of the Cinereous vulture (KCHA (Korean Cultural Heritage Administration) 2004), the possibility of Pb exposure from the breeding site (Mongolia) or stopover area (China) should be considered because some individuals are likely to die at or upon arrival.

Annually more than 100 immature (1–3 year-old) Cinereous vultures that were pushed out of their primary feeding habitat (Mongolia) are likely dead by starvation in South Korea (Lee et al. 2006). Concurrently, Cinereous vultures tend to congregate around feeding stations near DMZ because artificial foods such as a raw/processed cold meat of pigs and cows have been provided for these vultures by local authorities. There is no evidence that this supply of food contains Pb shot, however, the supplementary foods were provided without proper management including analyses of contaminants and/or anti-inflammatory drugs. Various chemicals including non-steroidal anti-inflammatory drug (NSAID) have been considered diagnostic for the main cause of death in raptors (Lumeij et al. 1993; Kwon et al. 2004; Oaks et al. 2004; Hernández and Margalida 2008). Thus, it is necessary to verify whether the food items including artificial supplies for wild vultures are free from various contaminants and/or NSAIDs in South Korea.

Our findings indicated that most of the dead Cinereous vultures may be suffering from abnormally high Pb exposure, indicating a potentially significant source of mortality to the population. We suggest greater regulatory activity over the broad spectrum of this endangered species, such as regulation of contamination sources and proper food supplies with reliable quality control at feeding stations.

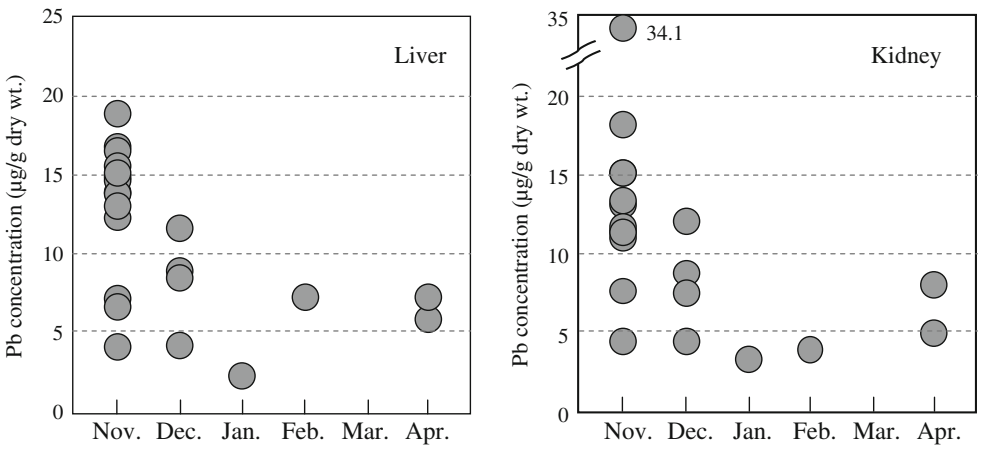

Fig. 1 Lead concentrations in liver and kidney of Cinereous vultures by sampling month

International collaboration for the conservation of the Asian population of Cinereous vultures should also focus on the ecological and physiological responses associated with chemical exposure.

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