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LEAD TOXICITY: CONSEQUENCES AND INTERVENTIONS IN AN INTENSIVELY MANAGED (*GYPS COPROTHERES*) VULTURE COLONY

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Abstract: The National Zoological Gardens of South Africa (NZG) is involved in the ex situ conservation of *Gyps coprotheres*, the Cape Griffon vulture (CGV) and houses 24 birds in a 100-yr-old aviary. Following the death of one vulture with high liver lead concentrations, an investigation was launched to ascertain the source(s) and consequences of lead toxicity in this breeding colony. Whole blood from 24 CGV, paint from the enclosure, water, and soil sampled at various locations within the enclosure were evaluated for their lead concentration, and data were gathered from NZG's medical records. The lead concentration in the paint, water, and enclosure soil was 5,100 μg/g, 0.5 μg/dl, and 72.48 ± 21.83 μg/g, respectively. The whole-blood lead concentrations were 56.58 ± 11 μg/dl. The breeding history of six pairs within the contaminated enclosure since 2002 showed 45 eggs laid, of which 44% were infertile and 24% successfully reared. The medical records revealed evidence of osteodystrophy despite adequate nutrition. As intervention measures, six birds were treated with Ca^{2+}EDTA and the topsoil inside the enclosure was replaced. As a result, the lead concentration in the enclosure soil dropped to14.74 ± 14.39 μg/g, and the whole-blood lead concentrations declined to 42.75 ± 11.64 μg/dl. It was concluded that lead concentrations in whole blood in excess of 100 μg/dl leads to clinical signs of lead toxicity in the CGV. Lower levels appear to interfere mainly with reproductive potential.

Key words: Lead, vultures, paint, *Gyps coprotheres*, calcium EDTA, soil

INTRODUCTION

In southern Africa, the Cape (Griffon) vulture (*Gyps coprotheres*; CGV) is an endemic species characterized as the heaviest vulture in Africa. On average, this species weighs 9 kg, and produces only one egg a year after reaching maturity at approximately 7 yr of age. The CGV typically nests on cliffs and tends to form life-long mating bonds, in a lifespan that in captivity has extended beyond 30 yr. The species is also listed by the World Conservation Union (IUCN) as vulnerable with only ±2,800 breeding pairs left in the wild. To this end, numerous organizations like the National Zoological Gardens of South Africa (NZG) have become involved in *ex situ* conservation. The NZG is located in the city of Pretoria, South Africa, and has been in existence since 1899 for display and breeding purposes. Recently, one of the NZG's 19-yr-old male CGV had intermittent signs of listlessness and anorexia, and subsequently died. Following necropsy and liver lead analysis of 51 μg/dl, a diagnosis of lead intoxication was made.

As a substance, lead is an inert heavy metal that plays no major role in the body's normal physiology and as a result becomes toxic when high concentrations occur within the body. In addition to acute toxicity, chronic toxicity can result following exposure to smaller concentrations of lead over a longer period of time, which can range from months to years. Sources of lead are varied, but commonly include lead-based paints, lead-rich soils, and plants grown in lead-rich soils. Other sources include lead pellets or plants contaminated by leaded petrol emissions.[4,8]

In captive populations, the most frequent of these lead sources comes through the consumption of carcasses that contain lead pellets or from lead-containing paints used in enclosures. For the latter, the exposure can occur directly via the ingestion of the lead flakes or indirectly via the soil into which the lead has leached.[3,12] Because no lead pellet was present in the digestive tract of the dead vulture, the enclosure was suspected as being the possible source of the intoxication. For this study, the paint, soil, water, and whole-blood lead concentrations of vultures within the enclosure were evaluated.

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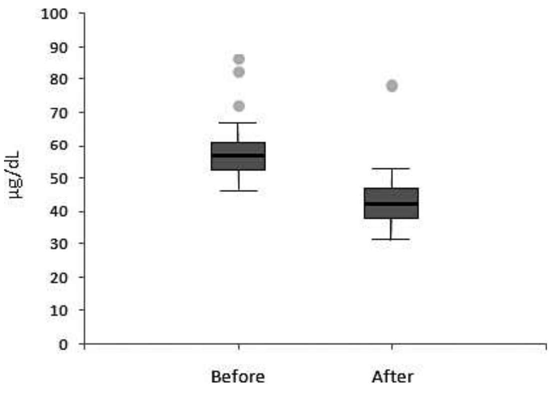

Figure 1. Whole-blood lead concentration (µg/dl) before and after intervention measures. The upper and lower border of the box represents the 95% lower and upper confidence levels, whereas the error bars represent mean ±SD. The center of the box indicates the mean. The dots represent the outliers.

MATERIALS AND METHODS

Description of study site

The NZG currently houses Cape Griffon vultures ($n = 25$) and African white-backed vultures (*Gyps africanus*; $n = 5$) in an enclosure consisting of a large freestanding steel frame and wired structure ($30 \times 20 \times 5$) that was more than 100 yr old. The aviary mimics a natural environment with a number of natural trees and a raised cliff with ledges suitable for breeding. The birds are fed twice weekly on chunks of fresh meat with bone, placed on a cement floor (2×3 m), although during feeding the birds routinely drag the meat onto the soil. The meat is specifically sourced to be free of lead pellets. Water is provided by means of a communal dam supplied by potable municipal water.

Husbandry

The NZG collated breeding data on the CGV vultures since 2002. The information is linked to the International Species Information System (ISIS), a global database in which more than 600 zoos participate annually. A hands-on breeding program is followed where the first egg laid by a breeding pair is removed for artificial incubation and subsequently hand reared. When a breeding pair produces more than one egg in a particular breeding season, the second egg is left for parental rearing.

Hand rearing involves the feeding of the chicks initially with mouse pinkies for a day or two, then mouse fuzzies and adult mice until they go over to an adult diet of day-old chicks with pieces of cut meat, supplemented with calcium for a few feedings. The calcium supplement used was Predator Supplement (Healthtech, Healthtech House, Midrand, Gauteng, 2000, South Africa.) at the recommended dose of 30 g/4 kg of meat, not including the bone. The powder was inserted into a cut made in the meat. The adult diet consists of chunks of meat with bone, such as racks of ribs, pelvises, and legs of a carcass.

A medical database was maintained of all treatments and necropsies of the NZG animal collection. Mining of this database revealed two records of suspected lead toxicity in juvenile vultures. In January 2009, a 4-mo-old male CGV on the nest and being fed by its parents underwent euthanasia because of severe bone deformities, despite being intensively treated for calcium deficiency. A second record was reported by a nongovernmental organization, the Vulture Programme (VULPRO) of the Rhino and Lion Wildlife Conservation nonprofit organization, which hand-reared a chick on behalf of the NZG, also documented bone abnormalities. Both cases are further discussed below.

Sampling and analysis

The approach was to collect blood samples from all the birds in the enclosure at different intervals. The sampling was approved by the Ethics and Scientific Committee of the NZG following the guidelines of the South African code of practice for research on animals. Twenty-four CGV were sampled in December 2008 and December 2009. Blood was collected from the tarsal vein with 5-ml syringes and transferred into EDTA tubes. All the blood samples were analyzed for lead content by a commercial medical clinical pathology laboratory (Ampath, Pretoria, South Africa). Samples were analyzed with the use of standard atomic absorption spectroscopy.

In addition, samples of the paint from the structure and soil samples at various distances in and around the feeding area in the enclosure were taken in January 2009. Soil samples were collected 5 and 10 m away from the enclosure, from the feeding area, from the water pipes entering the vulture drinking dam, at the artificial cliffs, and at the dam itself (Fig. 1). The soil in the enclosure was retested in August 2010 to determine whether lead levels in the soil were increasing. A water sample was also analyzed during the second sampling, to confirm that the water source was free of lead. Samples were analyzed by the Onderstepoort Veterinary Institute (OVI; Pretoria, South Africa), which also used standard atomic absorption spectroscopy.

Mitigation

As a result of the high plasma lead concentrations, the NZG implemented the following treatment plan. Six birds with the highest concentrations were treated with intramuscular Ca^{2+}EDTA (Kyron Laboratories, Benrose, Johannesburg, 2000, Gauteng, South Africa) at the recommended dose of 40 mg/kg IM, daily for 5 days. In addition, the birds were temporarily removed from their enclosure and 1 m of soil was dug out of the enclosure and replaced with river sand from an area that has not been known to have been exposed to leaded paints or any other potential lead source.

RESULTS

Lead concentrations

Of the 24 CGV sampled in December 2008 and 2009, 1 bird in each period was a juvenile of 6 and 3 mo for the respective year. The 2008 fledgling had concentrations of 42 µg/dl; the other fledgling had a concentration of 60 µg/dl. For the yearly resampling, only 23 birds from 2008 were resampled, as 1 bird had died in the interim from an unknown cause.

For the year 2008, the birds had a mean lead concentration of 56.58 ± 11 µg/dl. The lead concentration in the paint was high at 5,100 µg/g, whereas the concentration in the water was less than 0.50 µg/dl. The six birds treated with intramuscular Ca^{2+}EDTA (Kyron Laboratories) showed a decline of whole-blood lead concentrations d to 42.75 ± 11.64 µg/dl, which represented an overall decline of 13.28 ± 20.21 µg/dl (Fig. 1). One year later, of the 23 birds retested in 2009, 20 showed a decline in blood lead concentration; 1 bird showed no change and the other 2 showed an increase of 30 and 3 µg/dl, respectively. When only the decrease in blood lead is compared between the birds that received Ca^{2+}EDTA (Kyron Laboratories) and the birds that were exposed to only habitat modification, the changes in the blood-lead concentrations were 27 ± 23 and 13.14 ± 17.43 µg/dl, respectively, with an average of 19.14 ± 14.44 for all birds. The overall change was highly significant, with the use of a paired *t*-test ($n = 20$, $P < 0.000$).

The lead concentrations within the enclosure declined following the placement of the clean river sand, representing a change of 72.48 ± 21.83 µg/g to 14.74 ± 14.39 µg/g. The lead concentrations outside the enclosure increased from 9.75 ± 4.03 to 12.9 ± 4.38 µg/g. The individual concentrations present within specific areas within the enclosure are presented in Figure 2.

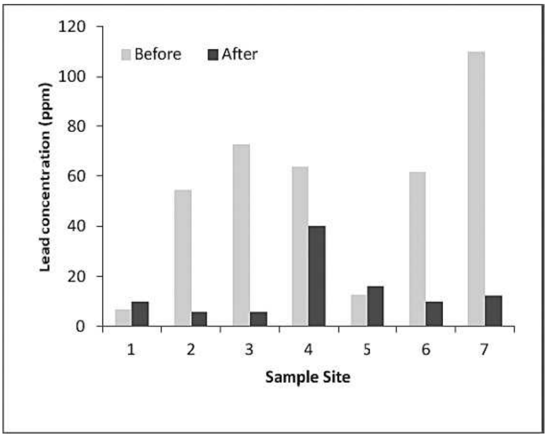

Figure 2. Concentration of lead in the soil at various sites before and after intervention. (**1**) 10 m outside the enclosure, (**2**) feeding area, (**3**) feeding area, (**4**) near pipes, (**5**) 5 m outside the enclosure, (**6**) cliff, (**7**) dam.

Breeding program

Since 2002, the CGV formed six breeding pairs and laid 45 eggs, at an average of 1.25 eggs/pair/year. Of these, 20 eggs were infertile (44.4%), 8 died in the embryonic stage (17%), 7 died within the first week of life (15.5%), and 11 were successfully reared on the nest (24%). Also included in the medical database is the information from one chick that was parent raised on the nest. It was taken off the nest in November 2008 for medical assessment when it was 2 mo old. The chick was depressed and not standing, with drooping wing and soft bones. The chick was diagnosed with severe metabolic bone disease suspected to be due to insufficient calcium supplementation. Calcium and Vitamin B complex + Oligo (Kyron Laboratories) treatment was immediately administered by intramuscular injection and calcium supplementation of the diet. One month after treatment, the chick developed a pathological fracture of the distal left humerus. Despite subsequently strapping of the wing, pathological fractures of the left and right wing were reported. Because of the permanent nonfunctioning of the wing, the chick was euthanized. Necropsy revealed brittle and thin cortices. Stored liver samples, made available for this study, were submitted to the OVI and yielded a lead concentration of less than 0.19 µg/g.

Figure 3. Radiographs from the VULPRO vulture chick hand raised, showing signs of severe osteodystrophy seen by the thin cortices of all the long bones, folding fractions (small arrows), bowing (large solid arrow), and green-stick fracture (large open arrow).

The male chick raised by the VULPRO developed osteodystrophy and pathological fractures (Fig. 3) despite being on mouse pinkies and fuzzies for the first 2 wk of its life, followed by a mice and rat diet, calcium supplementation, and daily half-hourly exposure to sunlight. Following the diagnosis of the fractures, the bird was treated at the Onderstepoort Veterinary Academic hospital with Ca^{2+}EDTA (Kyron Laboratories) for 2 wk as a prevention for possible lead intoxication). In addition, continued calcium supplementation, and immobilization of the wings through strapping to the body for 6 wk was performed. The bird made a full recovery following treatment, albeit with minor misalignment being present. The bird is currently in captivity and cannot fly.

DISCUSSION

This study clearly indicates that the CGV of the NZG have been exposed to high concentrations of lead. With the uniform level of toxicity, their exposure could only have resulted through a single common source; a contaminated enclosure was the most likely source for the birds of this study. Although the consumption of paint flecks may explain the high toxicity, exposure via the soil is the most likely cause of exposure. This can be confirmed from behavioral observations of the vultures during feeding time, as the vultures routinely drag their food across the ground as they tear out chunks to be swallowed. Further support for soil as the source of the intoxication is evident in the high lead concentrations of the two chicks. Being altricial, the chicks spend all their time on the cliffs inside the enclosure and as such are only exposed to lead from the food regurgitated by their parents and not the leaded paint.

The soil as a source of intoxication rather than lead-based paint is not unusual. Lead intoxication in children is believed to occur more commonly from soil than from paint.[10] In addition, the lead that originates from soil may be more toxic, due to superior bioavailability. In a study of pigs, lead in soil originating from paints was shown to be highly bioavailable at approximately 80% due to the solubility of the formed lead oxide in the acidic environment of the stomach.[7,11] This is in marked contrast to leaded pellets, which have a bioavailability of only 10%.[13] Although the bioavailability in birds has not been established, the presence of a highly acidic stomach contents in the vulture will most likely have absorptive patterns similar to that of the monogastric stomach of the pig.[9]

To reduce further exposure of the birds, the intervention measure of replacing the top layers of soil resulted in a direct decrease in the lead concentration within the enclosure and a reduction of the whole-blood lead concentrations in 20 of the 23 birds within the year. Nonetheless, the six birds treated with Ca^{2+}EDTA showed a superior response to just environmental modification. This is not surprising, as CA^{2+} EDTA is a known chelator of lead.[14] It physically binds whole lead in the blood, allowing it to be excreted at a higher level via the kidneys. The cause of failure of the other three birds to respond to adequate treatment is unknown. One possible reason could have been their ingestion of the paint fragments directly, as seen with albatross chicks.[2]

The lead concentration outside the enclosure, however, continued to increase. Although the decrease is easily explained by the introduction of new soil, the increase in the outside areas is more difficult to explain, as it is unlikely that the paint flecks migrated. The presence of a gradient from 5 to 10 m outside the enclosure does allow for a plausible explanation. With leaded paints, the contamination of the soil results in one of two manners—either directly by flecks of paint or indirectly by the paint flecks leaching soluble lead oxide in the soil. The latter would be free to move away from the enclosure with the water table, which explains the increases seen outside the enclosure.[11] Unfortunately, this implies that with time, the new soil inside the enclosure will be recontaminated as the lead leaches into it.

Despite the high whole-blood concentration of lead within the NZG birds, the levels did not appear to be sufficiently high to induce a full clinical toxicity. This would imply that the CGV is not as susceptible to lead toxicity as mammalian species. Their response was similar to that found in the griffon vulture (*Gyps fulvis*) for which it is speculated that blood concentrations above 100 μg/dl are required before overt clinical signs of toxicity develop.[5] This may not, however, be the case for subclinical toxicity. From published literature, it is recognized that subclinical lead toxicity occurs at concentrations ranging between 20 and 50 μg/dl, although a study on Californian condors (*Gymnogyps californianus*) suggested a lower limit of 10 μg/dl.[2] Effects would manifest as poor egg quality, egg fertility, and survivability, including abnormalities in chick bones despite the presence of healthy females and normal egg production,[1,6] all signs evident in this vulture population. It would therefore appear that the lead exposure in this current study caused a subclinical effect, as is evident from the poor hatchability of eggs despite the large number of eggs produced, i.e., 1.25 eggs/pair/year compared to the normal production in the wild with 1 egg/pair/year. The clinical signs of angular limb deformities identified in the two vulture chicks, from the NZG's medical database, may also have arisen from the lead exposure, as the chick treated with Ca^{2+}EDTA (Kyron Laboratories) treatment did recover.

CONCLUSION

The CGV of the NZG were exposed to high concentrations of lead in the soil. The lead most likely originated from the leaching paint flecks within the enclosure. Although the high lead concentrations had no overt clinical effect on the vultures, it did affect the breeding ability of the birds, as evident from the large degree of embryonic death/egg infertility. As a result, it should be considered that the toxic levels suggested for the griffon vulture by Garcia-Fernandez et al.,[5] where concentrations in excess of 100 μg/dl would result in clinical toxicity and concentrations from 20 to 100 μg/dl would be indicative of subclinical toxicity, are applicable for the CGV.

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